Metabolomics 2015

11th Annual International Conference of the Metabolomics Society
June 29-July 2, 2015
San Francisco Bay Area, California, USA

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Program Overview Sunday

	Sunday Harbour A	Harbour B	28-Jun Sandpebble
7:40 AM			
8:00 AM			
8:15 AM			
8:30 AM			
8:45 AM			
9:00 AM	**Metabolomics**	**NSF-JST workshop**	
9:15 AM	**Hackathon**	**Mtx for a Low Carbon Society**	
9:30 AM	Wohlgemuth, Schymanski	Okamuro, Sadaoka, Nishioka	
9:45 AM	*@ licenses: code, data, contents*	**NSF-JST workshop**	
10:00 AM		Qiu	
10:15 AM	break	Higashi	
10:30 AM	**Metabolomics**	Fine	
10:45 AM	**Hackathon**	break	
11:00 AM		**NSF-JST workshop**	
11:15 AM	*@ synchronizing MassBanks*	Fiehn	
11:30 AM	*MS and MS/MS workflows*	Ohta	
11:45 AM		Ono	
12:00 PM	lunch	lunch	
12:15 PM			
12:30 PM			
12:45 PM			
1:00 PM	**Metabolomics**	**NSF-JST workshop**	**BioCyc**
1:15 PM	**Hackathon**	Tsugawa	**Training Workshop**
1:30 PM			
1:45 PM	*@ Semantic Web/RDF/Sparql*	Putri	*(register via*
2:00 PM		Liao	*conference website)*
2:15 PM	break	break	
2:30 PM	**Metabolomics**	**NSF-JST workshop**	
2:45 PM	**Hackathon**	Jander	
3:00 PM		Schmelz	
3:15 PM	*@ R- packages*	Nikolau	
3:30 PM			
3:45 PM			
4:00 PM		Hyatt Atrium	
4:15 PM		**Early Career Member**	
4:30 PM		**Network**	
4:45 PM		**Reception**	
5:00 PM			
5:15 PM			
5:30 PM			
5:45 PM			
6:00 PM	**Agilent tour**		
6:15 PM			
6:30 PM			
6:45 PM			
7:00 PM			
7:15 PM			
7:30 PM			
7:45 PM			
8:00 PM			
8:15 PM			

Program Overview Monday

	Monday Regency	Grand BC	29-Jun Grand FG
7:40 AM 8:00 AM			
8:15 AM 8:30 AM 8:45 AM 9:00 AM 9:15 AM 9:30 AM	NIH Workshop **Pilot projects 1** * cancer * lung diseases	workshop *** data quality & pitfalls** *** study designs**	EMN workshop **Big Data**
9:45 AM		break	
10:00 AM 10:15 AM 10:30 AM 10:45 AM 11:00 AM 11:15 AM	NIH Workshop **Pilot projects 2** * central nervous system * other diseases	workshop *** compound annotation** *** open MS databases**	EMN workshop **Metabolic Pathways**
11:30 AM 11:45 AM 12:00 PM 12:15 PM 12:30 PM		luncheons **Bruker**	**Waters**
12:45 PM 1:00 PM 1:15 PM 1:30 PM 1:45 PM 2:00 PM	NIH Workshop **Pilot projects 3** * metabolic syndrome * kidney diseases	workshop *Frontiers in Metabolomics* *** advances in separations** *** stable isotope-assisted metabolomics** *** single cell metabolomics**	EMN workshop **Careers in Metabolism**
2:15 PM 2:30 PM	break		break
2:45 PM 3:00 PM 3:15 PM 3:30 PM 3:45 PM 4:00 PM 4:15 PM	workshop **Technology showcase**	break **NIH ring trial** * platforms used * compounds annotated * statistics	EMN workshop **Ethics in Research**
4:30 PM 4:45 PM		break	
5:00 PM 5:15 PM 5:30 PM 5:45 PM 6:00 PM	**plenary 1** **Mike Snyder, USA** Personalized Medicine: Dynamic global changes during environmental perturbations revealed using longitudinal multiomics profiling		
6:15 PM 6:30 PM 6:45 PM 7:00 PM 7:15 PM 7:30 PM 7:45 PM 8:00 PM 8:15 PM		**Welcome Reception** **poster session 1:** cancer metabolomic methods food & health respiratory diseases central nervous system diseases human diseases, other	

Program Overview Tuesday

	Tuesday 30-Jun		
	Regency	Grand BC	Grand FG
7:40 AM 8:00 AM 8:15 AM		breakfast session **Biocrates**	
8:30 AM 8:45 AM 9:00 AM 9:15 AM	**plenary 2** **Anne Osbourn, UK** Harnessing plant metabolic diversity		
9:30 AM 9:45 AM		break	
10:00 AM 10:15 AM 10:30 AM 10:45 AM 11:00 AM 11:15 AM	**cardiovascular &** **lung diseases**	**novel use of** **isotope labels**	**data** **processing**
11:30 AM 11:45 AM 12:00 PM 12:15 PM 12:30 PM		luncheons **Agilent**	**Thermo Scientific**
12:45 PM 1:00 PM 1:15 PM 1:30 PM 1:45 PM 2:00 PM 2:15 PM 2:30 PM		**poster session 2:** compound ID & databases environment & exposure healthy aging metabolic syndrome pathways & microbial metabolism human diseases, infections	
2:45 PM 3:00 PM 3:15 PM 3:30 PM 3:45 PM 4:00 PM	**cancer metabolism** **in human subjects**	**environmental &** **exposure studies**	**plant databases &** **plant systems biology**
4:15 PM 4:30 PM		break	
4:45 PM 5:00 PM 5:15 PM 5:30 PM 5:45 PM 6:00 PM	**pharma-** **metabolomics**	**imaging &** **MS-integration**	**pathways &** **microbial metabolism**
6:15 PM 6:30 PM 6:45 PM 7:00 PM 7:15 PM 7:30 PM 7:45 PM 8:00 PM 8:15 PM	Metabolomics Society **task group** meetings	Metabolomics Society **USA national affiliation** inauguration & task groups	Metabolomics Society **early career members** task groups

Program Overview Wednesday

	Wednesday Regency	 Grand BC	1-Jul Grand FG
7:40 AM		breakfast session	
8:00 AM		**Leco + Gerstel**	
8:15 AM			
8:30 AM	**plenary 3**		
8:45 AM	**Ben Cravatt, USA**		
9:00 AM	Mapping biochemical pathways in human disease		
9:15 AM	by integrated proteomics and metabolomics		
9:30 AM		break	
9:45 AM			
10:00 AM	**compound**	**diabetes &**	**repair metabolism**
10:15 AM	**identification**	**metabolic**	**novel pathways**
10:30 AM		**syndrome**	
10:45 AM			
11:00 AM			
11:15 AM			
11:30 AM		luncheons	
11:45 AM		**Metanomics Health**	**Sciex**
12:00 PM			
12:15 PM			
12:30 PM			
12:45 PM		**poster session 3:**	
1:00 PM		data processing	
1:15 PM		plants	
1:30 PM		metabolomic methods	
1:45 PM		human diseases, animals + insects	
2:00 PM		epidemiology	
2:15 PM		pharmametabolomics	
2:30 PM		pathways & microbial metabolism	
2:45 PM	**healthy**	**epidemiology**	**plant**
3:00 PM	**aging**	**in human health**	**metabolism**
3:15 PM			
3:30 PM			
3:45 PM			
4:00 PM			
4:15 PM		break	
4:30 PM			
4:45 PM	**compound ID &**	**diseases of the**	**mQTLs: genetics &**
5:00 PM	**databases**	**central nervous**	**metabolism**
5:15 PM		**system**	
5:30 PM			
5:45 PM			
6:00 PM			
6:15 PM			
6:30 PM		break	
6:45 PM			
7:00 PM			
7:15 PM			
7:30 PM	**Conference Dinner Party**		
7:45 PM			
-			
9:15 PM			

Program Overview Thursday

	Thursday Regency	Grand BC	2-Jul Grand FG
7:40 AM			
8:00 AM			
8:15 AM			
8:30 AM	**plenary 4**		
8:45 AM	**Zoltan Takats, UK**		
9:00 AM	Direct metabolic profiling of tissues, cell cultures and microbes		
9:15 AM	by ambient MS - from fundamentals to medical diagnostics		
9:30 AM		break	
9:45 AM			
10:00 AM	**metabolomics**	**food &**	**systems biology**
10:15 AM	**methods**	**health**	**of cancer**
10:30 AM			
10:45 AM			
11:00 AM			
11:15 AM			
11:30 AM			
11:45 AM	**Metabolon, John Ryals, USA**		
12:00 PM	Metabolomics and personalized medicine		
12:15 PM			
12:30 PM			
12:45 PM		break	
1:00 PM	**plenary 5**		
1:15 PM	**Jie Luo, PR China**		
1:30 PM	Forward-genetics-based interactive functional genomics and metabolomics		
1:45 PM			
2:00 PM	EMN Awards		
2:15 PM	Vendor Awards		
2:30 PM	Society Awards		
2:45 PM			**Thermo tour**
3:00 PM			
3:15 PM			
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9:15 PM			

WORKSHOPS

Monday, June 29th

(for NIH workshops 1-3, see ORALS abstracts)

8:15-9:45 a.m.

Regency **NIH workshop 1 "Cancer and Lung Diseases"** (for details, see ORALS 6-11)

Grand BC **Metabolomics 101 - From Experimental Design and Sample Preparation to Data Quality, Data Standards and Data Sharing**

Overall goals: In part 1, participants will learn the key concepts in establishing a metabolomics study, and how to design and complete a successful experiment. Part 2 will provide an overview of current practices, and educate new and experienced metabolomics researchers in the requirements for quality assurance and quality control. Part 3 will educate researchers in metabolomics data sharing, data standards, and data exchange.

Target audience: This is a 'General Practices' theme aimed at beginners starting in the metabolomics field, graduate students, post-doctoral researchers, principle investigators, and new investigators. Attendees will learn about experimental design, sample preparation, and metabolomics data sharing and standards.

Charmion Cruickshank-Quinn Samantha Bokatzian (U Colorado, Denver, USA)	Key concepts in establishing a metabolomics study, and how to design and complete a successful experiment
Dan Bearden (NIST, USA) Warwick Dunn (Birmingham, UK)	Overview of current practices and requirements for quality assurance and quality control.
Reza Salek (EMBL-EBI, UK) Steffen Neumann (IPB Halle, Germany) Philippe Rocca-Serra (U Oxford, UK)	Metabolomics data sharing, data standards, and data exchange

Grand FG **Early Career Member Network – "Setting sail in the sea of big data"**

Chairs: Nicholas Rattray (U Manchester, UK)
and Justin van der Hooft (U Glasgow, UK)

METABOLOMICS SOCIETY
EARLY- CAREER MEMBERS NETWORK

Big Data is defined in several ways over the last decade, but here we refer to large –omics data sets generated from metabolomics platforms. How to effectively handle Big Data sets, tidy up to remove unwanted noise, extract relevant information, and visualize Big Data sets in a meaningful manner are just a few of the topics that many scientists will encounter during analysis large omics data sets. Navigating your way through the oceans of data generated in large metabolomics studies is often disorienting and time consuming. In this workshop session we aim to discuss the importance of Big Data generation, how to handle Big Data, the existence of tightly regulated standards, and how to extract relevant information.

Douglas B Kell (U Manchester, UK)	Canonical Strategies for Analysing Tabular Metabolomics Data
Jeremy M. Shaver (Eigenvector Research, USA)	The Implementation of New High-Power Multivariate Analysis Tools and Preprocessing Methods for Metabolomics Data Analysis
Robert L Davidson (GigaScience, BGI Research, Hong Kong)	Open Data, Open Source: Preparing for Big Data in Metabolomic

10:00-11:30 a.m.

Regency NIH workshop 2 "CNS and other diseases" (for details, see ORALS 12-17)

Grand BC Compound Identification in Metabolomics Workshop

Metabolomics aims to measure the occurrence and concentration of as many metabolites as possible in a given sample. Chromatographic and spectroscopic methods such as LC-MS, GC-MS or NMR are most commonly used. In this workshop, the major focus will be on MS as a high throughput method. In most metabolomics experiments, the scientist is faced with a large number of features in the measured spectra, for which the structural identity of the underlying compounds are unknown. Mass spectral libraries provide an important source of reference information as a first step and an overview of the main resources as well as new developments in MassBank and other libraries will be given. Computer-assisted structure elucidation and dereplication provides further methods to aid the researcher in assigning likely structures to those unknowns, while the sheer volume of data created in typical metabolomics experiments calls for the development of robust and reliable high-throughput methods to speed up the identification process.

In this workshop we provide an overview of existing methods and demonstrate their applications and outline future developments. Ideas and discussions will be collected by the participants during the workshop in a shared document (http://goo.gl/LUNZQ5) to end up in a blog article/metabonews spotlight. Intended audience:

Metabolomics practitioners of all career stages with an interest in computer-assisted compound identification in metabolomics

Tomas Pluskal (Okinawa Inst Sci Technol, JP)	Data acquisition and pre-processing: Prerequisites for compound identification
Emmay Schymanski (EAWAG, CH)	Overview of different MS resources.
Masanori Arita (Natl. Genetics Inst., JP)	Toward a true reference: establishing spectral standards
Oliver Fiehn (UC Davis, USA)	MassBank of North America: an open resource for sharing mass spectra and metadata
Steffen Neumann (IPB Halle, DE)	Using compound databases and in-silico fragmentation to identify unknown compounds
Christoph Steinbeck (EMBL-EBI, UK)	De-novo compound identification: Computer-assisted structure elucidation of unknown compounds
Warwick Dunn (U Manchester, UK)	Highlights of CASMI 2014
Eoin Fahy (UC San Diego, USA)	Metabolite data storage, browsing and identification on the Metabolomics Workbench
	Discussion

10:00-11:30 a.m.

Grand FG **Early Career Member Network – "Fluxomics, Pathway Analysis, and Metabolic Engineering for a Sustainable Future"**

Chairs: Sastia Putri (Osaka U, JP)
Biswapriya Misra (U Florida, USA)

A major goal of metabolomics research is to provide tools to engineer cellular metabolism to benefit humanity for basic and specialized needs as a means of sustainable future. To achieve this beyond obtaining snapshots of the metabolic state of organisms, a comprehensive understanding of metabolic pathways and networks, shared metabolic intermediates, and metabolic flux in organelles, cells and the entire organism is required. Thus, a fluxomics and systems biology understanding will enable synthetic biologists to engineer metabolic pathways of interest in native systems and heterologous organisms, thereby working towards a sustainable future.

Uwe Sauer (ETH Zurick, CH)	Metabolomics as a hypothesis generator
James Liao (UCLA, USA)	Ensemble Metabolic Modeling
Akihiko Kondo (Kobe University, JP)	Metabolic engineering for the development of microbial cell factories based on systems biology approaches

12:45-2:15 p.m.

Regency **NIH workshop 3 "Metabolic syndrome and kidney diseases"**
(for details, see ORALS 18-23)

Grand FG **Early Career Member Network – "Careers in Metabolomics"**

Chairs: Gabriel Valbuena (Imperial College London, UK)
Jennifer Reid (The Metabolomics Innovation Centre, CA)

With the growth of metabolomics as a field of research, it becomes more important to consider the range of options available when it comes to future careers. In this workshop, we will explore pathways to different careers within Metabolomics, including tracks in academe, government, and industry. This will include a discussion of current trends in the field that may provide new and nontraditional career tracks, pathways to careers taken by panelists in the field, as well as tips and advice for early career researchers exploring future career options.

Andrew Patterson (PSU, USA)	How to become an Assistant Professor
Baljit Ubhi (Sciex, CA)	What is it to work in the MS industry?
David Balshaw (NIH / NIEHS, USA)	Perspective on Careers in a Research Funding Agency
Mike Wilson	Perspective on Careers in Launching a Startup Company

12:45-2:45 p.m. Grand BC Frontiers in Metabolomics

A general introduction to major challenges of current metabolomic initiatives including emerging technologies suited to address these current limitations.

topic 1 "Advanced separation techniques"
Philip Britz-McKibbin (McMaster U, CAN)

Despite the main focus on NMR and MS-based technologies for comprehensive metabolite profiling, high efficiency separation techniques play critical roles in resolving complex sample mixtures as required for accurate quantification and reliable identification of biologically or clinically relevant metabolites. This workshop will provide an overview of the major principles governing modern gas, liquid or SFC-phase separations based on chromatography, capillary electrophoresis and ion mobility spectrometry. New column technologies and robust methodologies that enhance peak capacity, selectivity and sample throughput will be discussed with special emphasis on their suitability for large-scale clinical or epidemiological investigations. Complementary separation modes and multidimensional separations that enhance metabolome coverage will also be examined, including in silico approaches for prediction of solute retention/migration behavior in support of MS/MS characterization. The main objective of the workshop is to highlight that separations provide several "value-added" benefits to metabolomics in comparison to direct-infusion MS studies as required for biomarker discovery.

This workshop is mainly aimed at a broad audience, including new and experienced users in metabolomics who are familiar with MS or NMR, but with perhaps experience in only one separation mode (typically reversed-phase 1D LC). The aim of the workshop is to provide participants a set of tools for appropriate selection and coupling of orthogonal separation modes to MS, including tips on ways to ensure good method robustness.

topic 2 Stable Isotope assisted strategies.
Rainer Schumacher (BOKU - U Vienna, AU)

The use of stable isotopes such as ^{13}C, ^{15}N, ^{18}O or ^{34}S has developed into a dynamic field of research, which offers great potential to tackle major existing challenges in metabolomics. Many novel strategies, workflows and bioinformatics tools have been developed in the last few years, which make use of labeled biological samples, tracers or derivatizing reagents for both targeted as well as untargeted metabolomics applications.

With respect to untargeted approaches, labeling-specific isotope patterns show high potential to be exploited for improved coverage of the global metabolic composition of a biological sample or the detection of novel metabolic pathways. In addition, more accurate quantification and improved metabolite annotation can be achieved by stable isotopic labeling. With respect to tracer metabolism, mass isotopomer distributions can be systematically evaluated to probe the dynamics of metabolic processes or to screen for novel tracer derived metabolites in the biological system under investigation.

topic 3 Single cell level analysis
Alfredo J. Ibanez (ETH Zurich, CH)

In recent years, many analytical techniques can perfectly address the needs for singlecell metabolomic studies. Furthermore, not only targeted metabolomic studies can be performed, but also multimodal analysis seems to be within reach. However, despite their promising potential, the idea of routine single-cell level metabolomics studies has not been fully accepted until now. This part of the workshop will cover

(a) State-of-the-art: overview of (i) Single-cell level metabolomics (Why is it needed?); (ii) Commercially available technologies capable of single-cell level metabolomics? (How is it performed?)

(b) New innovations and research: overview of new developments in the field of single-cell level metabolomic studies, with a particular focus on the analytical community efforts (in the fields of microfluidics, microarrays, imaging, etc.). including new data processing algorithms to improve our ability to better monitor biologically significant data by de-convoluting it from technical/analytical noise, as well as sample (cell) handling and quenching procedures that conserve the original metabolome of the cell, while being compatible with the requirements for ultrasensitive detection.

(c) Introduction of guidelines ("golden rules") and validation steps for improving the acquirement of metabolomic data at the single-cell level.

2:45-4:30 p.m. Regency

Technology Showcase: What are we eating? "

Industry leaders are invited to showcase their solutions for metabolomics data acquisition and data processing. **How can we assess the metabolome?**

Using three different mixed food plates, extracted homogenates were sent to the conference sponsors who were asked to apply their technologies and demonstrate, what these technologies can do?
How can we resolve the enormous complexity of metabolomes? When we identify compounds: How sure are we? When we process data: How do we set thresholds? How do we handle missing values?
How do we handle false positives, false negatives? And how do we assess quantification, precision, accuracy and overall differences?

Foods were chosen because they are the gateway to health and disease (and they certainly link plant, microbial and human metabolism!) **This is not a competition**. Foods are so complex, that many technologies are needed. There is no single 'true' answer – but many good approaches!

Chair: Gary Siuzdak, Scripps, USA
Food samples: Arpana Vaniya , West Coast Metabolomics Center, UC Davis, USA
Dr Nancy Keim, USDA, ARS, Western Human Nutrition Research Center, Davis, USA

Presenters:
Agilent
Bruker
Leco Gerstel
Sciex
Thermo Scientific
Veritomyx
Waters

3:00 – 4:30 pm

Grand BC NIH Metabolomics Ring Trial

In addition to genomics, transcriptomics or proteomics, untargeted metabolomics aims to establish itself as a standard enabling technology in pharma, nutrition or diagnostics R&D. In order to be used for widespread applications, metabolomics needs to improve its perceived level of reproducibility, robustness and overall value. This is particularly important for healthcare applications. The lack of common standards used for global metabolomics across commercial vendors, as well as academic institutions, remains as a major hurdle to routine use of the technology in healthcare research. A series of precompetitive ring trials could gradually increase standards of reporting across labs and enhance capabilities in metabolomics service laboratories, leading to wider usage of this enabling technology for biomarker discovery and validation.

David Balshaw, NIH/NIEHS, USA
Introduction to the NIH Common Fund program and the NIH metabolomics resource cores, including services, training, standards, technology development components, and data center.

Andrew Patterson, PSU, USA
Overview of the ring trial origins and development of specifics that were recommended by the NIH Common Fund advisory committee.

Arthur Edison, U Florida, USA
Publicly available protocols and unique capabilities of the NIH Common Fund metabolomics cores.

Eoin Fahy, UC San Diego, USA
Plans for analysis of the ring trial data

2:45 – 4:15 pm

Grand FG Early Career Member Network – "Research Integrity: How to not fake your data

Chairs: Thomas Payne (Imperial College London, UK)
= David Liesenfeld (German Research Cancer Center, DE)

In recent years, the number of scientific misbehaviors have spiked, with an increase in retractions of scientific articles compared to the first decade of the 21st century. Scientific misconducts are manifold, from falsification, fabrication ("cooking"), and distortion of data to selective reporting, plagiarism, and authorship manipulations. In this workshop session, we aim to present examples of scientific misconduct (from serious misbehaviors to questionable practices) in metabolomics or related research areas. Even more importantly, this session will point towards guidelines to avoid these malpractices, present consensus statements for the metabolomics community (i.e. the Metabolomics Standards Initiative) and give insights into good scientific research practices from an industrial perspective.

Roy Goodacre (U Manchester, UK)	Scientifics ethics: objectivity versus subjectivity?
Philipp Rocca-Serra (BOKU - U Vienna, AU)	The limits of data sharing: - Is data retention just scientific malpractice or can it be justified?
Oliver Schmitz (Metanomics, DE)	Metabolomics Research Integrity - The Industry Perspective

ORALS

(including NIH Monday workshop 1-3 oral presentations)

oral 001	50 min	**Keynote Monday 5:00 pm**	Michael Snyder

Personalized Medicine: Dynamic global changes during environmental perturbations revealed using longitudinal multiomics profiling

Wenyu Zhou, Stanford University, Stanford, US
Brian Piening, Stanford Univeirsty, Stanford, US
Kevin Contrepois, Stanford, CA, US
LIhua JIang, Stanford University, Stanford, US
Gucci Gu, Stanford Univeristy, Stanford, US
Shana Leopard, Jackson Laboratory, Farmington, US
Candice Allister, Stanford Univeristy, Stanford, US
Collen Craig, Stanford University, Stanford, US
Tejas Mishra, Stanford University, Stanford, US
Dalia Perelman, Stanford Univeristy, Stanford, US
Denis Salins, Stanford University, Stanford, US
Erica Weinstock, Jackson Laboratory, Farmington, US
Justin Sonnenburg, Stanford Univieristy, Stanford, US
George Weinstock, Jackson Laboratory, Farmington, US
Tracey MacLaughlin, Stanford University, Stanford, US
Michael Snyder, Stanford University, Stanford, US

Understanding health and disease requires a detailed analysis of both our DNA and the molecular events that determine human physiology. We performed an integrated Personal Omics Profiling (iPOP) on 70 healthy and prediabetic human subjects over periods of viral infection as well as during controlled weight gain and loss. Our iPOP integrates multiomics information from the host (genomics, epigenomics, transcriptomics, proteomics and metabolomics) and from the gut microbiome. For the metabolomics we optimized a LC-MS platform for detailed analysis of blood and urine. Longitudinal multiomics profiling reveals extensive dynamic biomolecular changes occur during times of perturbation, and the different perturbations have distinct effects on different biomolecules in terms of the levels and duration of changes that occur. Overall, our results demonstrate a global and system-wide level of biochemical and cellular changes occur during environment exposures.

oral 002	50 min	**Keynote Tuesday morning**	Anne Osbourn

Harnessing plant metabolic diversity

Anne Osbourn, John Innes Centre, Norwich, GB

Plants produce a tremendous array of natural products, including medicines, flavours, fragrances, pigments and insecticides. The vast majority of this metabolic diversity is as yet untapped, despite its huge potential value for humankind. So far research into natural products for the development of drugs, antibiotics and other useful chemicals has tended to focus on microbes, where genome sequencing has revolutionised natural product discovery through mining for gene clusters for new metabolic pathways. Identifying novel natural product pathways in plants is extremely difficult because plant genomes are much larger and more complex than those of microbes. However, the recent discovery that genes for some types of plant natural product pathways are organised as physical clusters is now enabling systematic mining of plant genomes in the quest for new pathways and chemistries. Improved understanding of the genomic organization of different types of specialized metabolic pathways will shed light on the mechanisms underpinning pathway and genome evolution. It will further open up unprecedented opportunities for exploiting Nature's chemical toolkit by providing grist for the synthetic biology mill.

oral 003	50 min	**Keynote Wednesday morning**	Benjamin Cravatt

Mapping biochemical pathways in human disease by integrated proteomics and metabolomic

Benjamin Cravatt, The Scripps Research Institute, La Jolla, US

Genome sequencing projects have revealed that eukaryotic and prokaryotic organisms universally possess a huge number of uncharacterized proteins. The functional annotation of these proteins should enrich our knowledge of the biochemical pathways that support human physiology and disease, as well as lead to the discovery of new therapeutic targets. To address these problems, we have introduced chemical proteomic and metabolomic technologies that globally profile enzyme activities in complex biological systems. Prominent among these methods is activity-based protein profiling (ABPP), which utilizes active site-directed chemical probes to determine the functional state of large numbers of enzymes in native proteomes. In this lecture, I will describe the application of ABPP to discover and functionally annotate enzyme activities in mammalian physiology and disease. I will also present competitive ABPP platforms for developing selective inhibitors for poorly characterized enzymes and show how these inhibitors, when combined with metabolomics, are powerful tools for assigning functions to enzymes in biological systems, including enzymes linked to human disease. Finally, I will discuss ongoing opportunities and challenges that face researchers interested in assigning functions to proteins in the genome era and emphasize how Finally, I will discuss ongoing opportunities and challenges that face researchers interested in assigning functions to proteins in the genome era and emphasize how chemoproteomics-guided pharmacology can play a central role in achieving this goal. Chemoproteomics-guided pharmacology plays a central role in defining functions to proteins linked to human diseases.

oral 004	50 min	**Keynote Thursday morning**	Zoltan Takats

Direct Metabolic Profiling of Tissues, Cell Cultures and Microorganisms by Ambient Mass Spectrometry - From Fundamentals to Medical Diagnostics

Zoltan Takats, Imperial College London, London, GB

While the recent development in the field of chromatographic and mass spectrometric techniques solved a number of long-standing problems in metabolomics (as well as in proteomics), the in-vivo chemical analysis of biological organisms is still a challenge for analytical and biological chemists. The advent of ambient mass spectrometric methods a decade ago gave new momentum to this area and the technical advancements of the last few years eventually seem to provide valid solutions to this problem. The in-vivo analysis is expected to shed light to metabolic processes with greater accuracy and it's also expected to facilitate the development of a new generation of medical diagnostic/patient stratification techniques. The presentation is aimed to cover a number of applications including the Desorption Electrospray Ionization - Mass Spectrometric (DESI-MS) analysis of tissues both in- and ex-vivo, using standrad DESI source (Prosolia) coupled to Q-TOF (Waters) and Orbitrap (Thermo) mass analysers. DESI-MS was performed on native skin surface, mucosal swabs and histological tissue sections. Alternatively to DESI, Rapid Evaporative Ionization Mass Spectrometry (REIMS) was used for the in-vivo and ex-vivo analysis of tissue and biological fluid specimens. REIMS analysis was performed by using the direct combination of standard electrosurgical equipment (Covidien) and Q-TOF mass spectrometry (Waters). REIMS results were validated by medical imaging (MRI, US) and histology. REIMS was also used for the high-throughput lipidomic profiling of bacteria and pathogenic fungi. DESI-MS is capable of the direct, often in-vivo analsis of biological systems. In it's simplest form, in-vivo DESI-MS was used for the analysis of human skin and squamous epithelium. Results revealed that the approach gives information both on the localised physiological/pathological processes including microbial activity, autoimmune processes, etc. and organism-level biochemical alterations. DESI was successfully used to characterize inflammatory processes with different aetiology and changes in the composition of the microbiome in various diseases. Furthermore, DESI was also successfully used for the detection of xenobiotics and their metabolites in a non-invasive manner. DESI-based mass spectrometric imaging of tissue sections provided data showing high level of histological specificity, going significantly beyond the level of morphological classification of tissues. Status of a number of well-established prognostic cancer biomarkers was successfully determined using untrageted DESI-MS imaging. Based on the success of DESI- (and MALDI-) MSI based lipidomic phenotyping of cancer patients, REIMS technique was developed for the intrasurgical (or intra-interventional) identification of tissues. REIMS technology was successfully used for the real-time analysis and classification of various human tissues including multiple different types of cancer. Tissue identification performance was found to be in the range of 95-99%, validated by post-interventional histopathology. Since REIMS is based on the

direct combination of a surgical technique (electrosurgical diathermy) with MS detection, the technology gives a convenient solution for the problem of in-situ tissue identification. REIMS technology can also be used for the analysis of arbitrary types of biomass including bacterial or fungal pathogens. A spectral library comprising >20,000 REIMS spectra of pathogens was constructed and used for the identification of pure colonies. The database was also used to derive taxon-specific conservative biomarkers, which enable the selective detection of bacterial and fungal pathogens in complex human tissue and biofluid matrices, as it was subsequently proven by DESI-MS. The discipline of in-vivo MS was established, giving new insights to tissue metabolism and opening new opportunities for medical diagnostics.

oral 005	50 min	**Keynote Thursday afternoon**	Jie Luo

Recent advance in forward-genetics-based interactive functional genomics and metabolomics

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Plants produce a vast array of chemically and biologically different compounds. These compounds are not only important for plant themselves and their interactions with the environment, but also provide indispensable resources for humans as sources for nutrition, energy, and medicine. Understanding the genes involved in metabolism and dissection of the metabolic pathway are essential to improve plant adaptation to environmental stresses, to improve food quality, and to increase the yield of major crops such as rice and maize through metabolomics-based breeding. Here we report the genetic studies of crop metabolomes combining both linkage and association analyses with comprehensive metabolic profiling. This strategy provides a powerful tool for large-scale interactive gene-metabolite annotation and identification, pathway elucidation and knowledge about crop improvement. Samples from either the recombinant inbred line (RIL) or a natural population were collected for high through-put metabolic profiling using a LC–electrospray ionization (ESI)-MS/MS system. Quantification of metabolites was carried out using a scheduled multiple reaction monitoring (MRM) method. We also sequenced the individual accessions or lines of rice and maize using the Illumina HiSeq 2000 system and high ultrahigh-density genetic map (13) was generated for both RIL and natural populations. Quantitative trait locus (QTL) mapping or genome-wide association study (GWAS) were then performed using levels of metabolites as traits, e.g. metabolic traits. Candidate genes underlying the metabolic traits that that are of physiological and/or nutritional importance were then mined and verified by integrated molecular and biochemical approaches. Application of a newly developed widely-targeted metabolomics strategy [1] simultaneously detected hundreds of both primary and secondary metabolites in rice and disclosed a number of subspecies-specific metabolites that may reflect, as well as affect the subspecies differentiation of rice. Distinct and overlapped accumulation was observed and complex genetic regulation of metabolism was revealed in two different tissues by subsequent metabolic QTL (mQTL) mapping [2] and metabolic genome-wide association study (mGWAS) [3]. Hundreds of loci with high resolution and large effects were uncovered. Interactive gene/metabolite identification/annotation was facilitated for both functional genomics and metabolomics. Data mining revealed a large number of candidate genes underlying metabolites that are of physiological and agronomical importance, and seven of them were experimentally verified. Based on these findings, comprehensive forward-genetics-based metabolic pathways were constructed. Similar approaches were also applied to the understanding of maize kernel metabolome [4]. Furthermore, comparative mGWAS between rice and maize resulted in greatly increased power and resolution in both species. In addition, parallel metabolic and phenotypic GWAS identified new candidate genes responsible for both metabolic and morphologic traits such as grain color and width, revealing direct linkage between the metabotype and the phenotype. Our study not only reveals novel biochemical and genetic insights of important aspects of plant and human such as development, stress resistance, and nutrition/health-promoting, but also provides vast amount of high-quality data for further understanding plant metabolome which may help bridge the gap between the genome and phenome. The strategy describes here is a powerful tool for large-scale gene identification, pathway elucidation, and for knowledge-based crop genetic improvement [5].

Ref: [1] Chen et al, Mol Plant, 2013; [2] Gong et al, PNAS, 2013; [3] Chen et al, Nat Genet, 2014; [4] Wen et al, Nat Commun, 2014; [5] Luo, Curr Opin Plant Biol, 2015. The strategy combining genetics, genomics and metabolomics as illustrated here provides a powerful tool for interactive functional genomics and metabolomics.

oral 006	15 min	NIH cancer * lung diseases	Jiannong Li

Proteo-metabolomic dissection of small cell lung cancer using activity based protein profiling and metabolomics profiling

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Small cell lung cancer (SCLC) is poorly differentiated neuroendocrine malignancy which is characterized by poor prognosis and early metastatic dissemination. To date, there are no significant improvements in outcome over platinum-etoposide chemotherapy for SCLC patients. Early detection biomarker strategies and novel therapeutic target discovery is understudied yet remains an important goal. We hypothesized that an assessment of the ATP binding proteome in SCLC coupled with metabolomics could deliver new insights into the disease and nominate both biomarkers and therapeutic targeting strategies. We performed activity-based protein profiling (ABPP) and metabolites profiling in lung cancer cell lines and human tissues. ABPP profiling was performed using desthiobiotin-ATP probe which is directed against the active sites of enzymes.The modified peptides were analyzed by LC-MS/MS and quantified using MaxQuant. The differentially expressed peptides between groups were identified using two-sample Wilcoxon adjusting rank sum test. For metabolites profiling, cell and lung extracts were analyzed by Ultra Performance Liquid Chromatography-Time-of-Flight-Mass Spectrometry (UPLC-TOF-MS). The data were processed using Waters' Progenesis QI software and subjected to statistical and multivariate analysis (e.g., PCA, OPLS-DA) using the SIMCA (Umetrics) software to determine the metabolites that best separate the groups based on inspection of loadings and VIP plots. We profiled the ATP-binding proteome of 18 SCLC and 18 non-SCLC (NSCLC) cell lines. We identified 6937 peptides (2319 proteins), of which 3891 peptides (1543 proteins) were differentially expressed. Several pathways related to metabolism, such as purine biosynthesis and glycolysis / gluconeogenesis, were enriched in SCLC cells compared to NSCLC. These results led us to perform broad spectrum UPLC-TOF-MS metabolomics on ten SCLC and ten NSCLC cell lines. Multivariate analysis demonstrated distinct metabolite profiles for SCLC and NSCLC. Over 100 metabolites with variable importance to projection greater than 1 contributed to the differentiation of the two groups. These included metabolites related to purine metabolism such as inosinic acid and adenosine monophosphate enriched in SCLC cell lines and suggested a connection between our proteomics and metabolomics results. We applied the same approaches to perform the ABPP and metabolite profiling in human lung tissues (7 SCLC, 7 NSCLC and 7 normal lung). ABPP combined with LC/MS/MS identified 17072 peptides (4445 proteins) - 38 proteins are significantly overly expressed in SCLC tissues compared with that of NSCLC and normal tissues. Of these 38 proteins, metabolic enzymes such as ACYP1, CKB, CKM, MTHFD1 and RRM1 were identified. In tissue metabolite profiling, we identified 92 annotated metabolites significantly different between disease (SCLC+NSCLC) and normal tissue. We found 6 metabolites (adenosine monophosphate, 3'-AMP, 8-Oxo-dGMP, Uridine diphosphate-N-acetylglucosamine, Pantothenic acid and FAPy-adenine) enriched in SCLC tissue consistent with our finding at the cell level. In conclusion, ABPP and metabolic profiling identifies distinct enzyme and metabolite profiles in SCLC cells and tissues. Ongoing work aims to integrate ABPP and metabolite profiling using different modeling such as Bayesian network analysis to jointly characterize the key pathways and constituent components in SCLC. We will update our finding at the meeting. Funded by NIH grants 1R21 CA169979-01A1 (E.B.H) and 1U24DK097193. Integrating proteomics and metabolomics has the potential to identify novel biomarkers and therapeutic targets in diseases.

oral 007	15 min	NIH cancer * lung diseases	Ernst Lengyel

A Systematic Analysis of Bidirectional Metabolomics Changes upon Interaction of Cancer Cells with Adipocytes

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Small cell lung cancer (SCLC) is poorly differentiated neuroendocrine malignancy which is characterized by poor prognosis and early metastatic dissemination. To date, there are no significant improvements in outcome over platinum-etoposide chemotherapy for SCLC patients. Early detection biomarker strategies and novel therapeutic target discovery is understudied yet remains an important goal. We hypothesized that an assessment of the ATP binding proteome in SCLC coupled with metabolomics could deliver new insights into the disease and nominate both biomarkers and therapeutic targeting strategies. We performed activity-based protein profiling (ABPP) and metabolites profiling in lung cancer cell lines and human tissues. ABPP profiling was performed using desthiobiotin-ATP probe which is directed against the active sites of enzymes.The modified peptides were analyzed by LC-MS/MS and quantified using MaxQuant. The differentially expressed peptides between groups were identified using two-sample Wilcoxon adjusting rank sum test. For metabolites profiling, cell and lung extracts were analyzed by Ultra Performance Liquid Chromatography-Time-of-Flight-Mass Spectrometry (UPLC-TOF-MS). The data were processed using Waters' Progenesis QI software and subjected to statistical and multivariate analysis (e.g., PCA, OPLS-DA) using the SIMCA (Umetrics) software to determine the metabolites that best separate the groups based on inspection of loadings and VIP plots. We profiled the ATP-binding proteome of 18 SCLC and 18 non-SCLC (NSCLC) cell lines. We identified 6937 peptides (2319 proteins), of which 3891 peptides (1543 proteins) were differentially expressed. Several pathways related to metabolism, such as purine biosynthesis and glycolysis / gluconeogenesis, were enriched in SCLC cells compared to NSCLC. These results led us to perform broad spectrum UPLC-TOF-MS metabolomics on ten SCLC and ten NSCLC cell lines. Multivariate analysis demonstrated distinct metabolite profiles for SCLC and NSCLC. Over 100 metabolites with variable importance to projection greater than 1 contributed to the differentiation of the two groups. These included metabolites related to purine metabolism such as inosinic acid and adenosine monophosphate enriched in SCLC cell lines and suggested a connection between our proteomics and metabolomics results. We applied the same approaches to perform the ABPP and metabolite profiling in human lung tissues (7 SCLC, 7 NSCLC and 7 normal lung). ABPP combined with LC/MS/MS identified 17072 peptides (4445 proteins) - 38 proteins are significantly overly expressed in SCLC tissues compared with that of NSCLC and normal tissues. Of these 38 proteins, metabolic enzymes such as ACYP1, CKB, CKM, MTHFD1 and RRM1 were identified. In tissue metabolite profiling, we identified 92 annotated metabolites significantly different between disease (SCLC+NSCLC) and normal tissue. We found 6 metabolites (adenosine monophosphate, 3'-AMP, 8-Oxo-dGMP, Uridine diphosphate-N-acetylglucosamine, Pantothenic acid and FAPy-adenine) enriched in SCLC tissue consistent with our finding at the cell level. In conclusion, ABPP and metabolic profiling identifies distinct enzyme and metabolite profiles in SCLC cells and tissues. Ongoing work aims to integrate ABPP and metabolite profiling using different modeling such as Bayesian network analysis to jointly characterize the key pathways and constituent components in SCLC. We will update our finding at the meeting. Funded by NIH grants 1R21 CA169979-01A1 (E.B.H) and 1U24DK097193. Integrating proteomics and metabolomics has the potential to identify novel biomarkers and therapeutic targets in diseases.

oral 008	15 min	NIH cancer * lung diseases	Ameeta Kelekar

Bcl-2 protein Noxa regulates cellular energetics and reveals novel pathways in cancer metabolism

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Cancer cells increase the consumption and metabolism of both glucose and glutamine and reprogram metabolic pathways to enhance biomass production. Glucose is often diverted to alternative pathways for the production of building blocks prior to the generation of pyruvate and lactate. However, a high glycolytic rate needs a steady supply of cytoplasmic NAD to be regenerated from NADH, a task that has historically been attributed to lactate dehydrogenase. This suggests that alternative pathways of NAD regeneration are being utilized. Furthermore, increased glutamine uptake and metabolism create an additional problem. The first two steps of glutaminolysis generate two molecules of free ammonia. How cancer cells mitigate the potential toxicity from this ammonia is currently unknown, and of immense therapeutic value. To address these questions, we used a T cell leukemia model in which over-expression of the pro-apoptotic Bcl-2 protein Noxa increases glucose consumption, extracellular acidification and proliferation. Noxahi cells metabolize the glucose through anabolic routes, including the pentose phosphate pathway. Thus, these cells represent a valuable tool for investigating metabolic adaptations in cancers. First, we carried out metabolic tracer analysis, with a [2H] labeled glucose that transfers the [2H] to NADH during glycolysis, to determine how Noxahi cells regenerate NAD. We also used [13C]-U-glutamine to trace its path via glutaminolysis and 15N-amide-glutamine to investigate the fate of free ammonia generated during glutaminolysis. Metabolites were identified using a combination of liquid chromatography and/or gas chromatography coupled with mass spectrometry. Tracer studies with [2H] glucose revealed that Noxahi cells prefer malate dehydrogenase to lactate dehydrogenase for regenerating cytoplasmic NAD. Tracer studies with [13C]-U-glutamine showed that the reductive carboxylation of glutamine is a significant source of malate in these cells and is able satisfy the demand for cytoplasmic malate for NAD regeneration. Unexpectedly, the experiment with [2H]- labeled glucose also revealed a significant increase in [2H] labeled aspartate in Noxahi cells. This aspartate could potentially be generated from early glycolytic intermediates or from a reaction that required NADH as a cofactor. To date, we have been unable to identify a pathway that would yield significant amounts of deuterated aspartate from glycolytic intermediates at 24 hours. There is, however, a reaction that requires NADH to produce aspartate. The enzyme aspartate dehydrogenase (ASPDH) utilizes the reactive hydrogen from NADH to generate aspartate from oxaloacetate. This reaction also utilizes free ammonia from the environment for the amine group of aspartate. As stated earlier, how cancer cells mitigate the potential toxicity that results from accumulation of free ammonia generated during glutaminolysis is currently not known, but is of much therapeutic interest. Our data suggest that aspartate from the ASPDH catalyzed reaction is exported out of the cell, contributing to the increased acidification. This pathway may represent a novel mechanism to package and excrete excess ammonia. We hypothesized, based on these observations, that siRNA targeted to ASPDH would decrease proliferation and/or promote cell death. We also hypothesized that any cancer cell type exhibiting dependence on glutamine should be sensitive to loss of ASPDH. Colorectal cancer cells transiently transfected with ASPDH siRNA transfected displayed significant decrease in cell viability and proliferation compared to control siRNA transfectants within 72 hours. Preliminary studies also showed an increase in detectable free ammonia in cells treated with ASPDH siRNA. Our studies indicate that cancer cells utilize a novel ASPDH catalyzed step for regenerating cytoplasmic NAD and counteracting ammonia toxicity.

oral 009	15 min	NIH cancer * lung diseases	Stephen Wedgwood

Metabolomics of Neonatal Pulmonary Hypertension

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Bronchopulmonary dysplasia (BPD) is a serious and common chronic lung disease of infancy. Pulmonary hypertension (PH) is a common complication of BPD that contributes to worse clinical outcomes and significantly increases the morbidity and mortality of preterm birth. PH is often not diagnosed in premature infants until symptoms emerge after many weeks of life, when the disease is advanced and associated with severe cardiac dysfunction. However early screening methods have not proven to be reliable predictors of PH and no biomarkers currently exist. A recent clinical study suggests that preterm infants with BPD and low birth weight are at increased risk of developing PH. The aim of this study is to identify novel biomarkers associated with BPD and PH. We combined an established rat model of BPD and PH, achieved by exposing newborn pups to 75% oxygen, with a model of postnatal growth restriction, achieved by increasing litter sizes from 10 to 17 pups. We hypothesized that growth restriction would exacerbate oxygen-induced PH. After 14d pups were sacrificed, heart and lungs snap frozen in liquid nitrogen and blood plasma collected and stored. Hearts and lungs were analyzed for evidence of PH. Lung and plasma samples were analyzed by the West Coast Metabolomics Center to detect primary metabolites by GC-TOF MS, and to detect complex lipids and oxylipins by UPLC-MS/MS. Archived cord blood from preterm infants with and without PH was also submitted to WCMC for analysis. Growth restriction or oxygen exposure independently induced PH as evidenced by right ventricular hypertrophy (RVH) and increased pulmonary artery wall thickness in 14d-old rats. Growth restriction and oxygen combined increased RVH and medial wall thickness further relative to either stress alone. Oxidant stress induces pulmonary hypertension via multiple mechanisms resulting from elevated levels of reactive oxygen species (ROS). Our preliminary data indicate that total ROS levels were elevated in the lungs of 14d-old rats exposed to growth restriction or oxygen, and ROS levels were elevated further in the lungs of rats exposed to both stresses. Metabolomics analysis of oxylipins in rat lung and blood samples indicate that growth restriction and oxygen increased the levels of distinct biomarkers of oxidant stress. For example, oxidation products of arachidonic acid include hydroxyeicosatetraenoic acids (HETEs) and dihydroxyeicosatrienoic acids (DiHETrEs); 5-HETE was increased in rats exposed to oxygen, 9-HETE was increased in rats exposed to growth restriction, while 5,6-, 8,9-, and 11,12-DiHETrEs were increased by oxygen or growth restriction and elevated further when both stresses were combined. Analysis of primary metabolites indicated that growth restriction and oxygen decreased lung and blood levels of citrulline, a precursor of arginine required for NO-mediated pulmonary vasodilation. Analysis of complex lipids indicated that in general, growth restriction decreased lysophosphatidylcholines while oxygen increased diacylglycerides and fatty acids in lung and blood. Analysis of cord blood from preterm infants with PH is currently underway. Our preliminary data indicate that growth restriction and oxygen may induce PH in neonatal rats via distinct and common signaling pathways. The generation of biochemical network maps and comparisons with data from patient cord blood samples may improve our knowledge of the underlying mechanisms that induce PH, and may identify novel biomarkers that predict the risk of developing PH before the disease is advanced. Our data provide for the first time a metabolomics profile of infants with PH and a novel model of PH.

oral 010	15 min	NIH cancer * lung diseases	Biao Hu

Metabolic changes in human fibroblasts isolated from Idiopathic pulmonary fibrosis patients

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Idiopathic pulmonary fibrosis (IPF) is characterized with excessive extracellular matrix deposition in the lung and changes attributed to associated hypoxia. There is mounting evidence to show that cells exposed to hypoxia alter

their metabolism to survive and this response to hypoxia has an imperative function in the pathogenesis of many diseases, as exemplified by ischemic disease following stroke or myocardial infarction and in tumorigenesis as well. Although studies with regulatory factors such as hypoxia inducible factor-1 (HIF-1), Poly [ADP-ribose] polymerase 1 and Sirtuin 1 etc. implied the potential for metabolic alterations in lung cells contributing to the progression of pulmonary fibrosis, direct metabolomic evidence is lacking. In this study, the glycolytic pathway and tricarboxylic acid (TCA) cycle metabolites in fibroblasts isolated from IPF patients or control subjects were analyzed by LC/MS, GC/MS and confirmed by ELISA assays. The results showed that most components of the glycolytic pathway and TCA cycle, including fructose 6-phosphate, glucose 6-phosphate, pyruvate, α-ketoglutarate (α-KG), fumarate, malate and oxaloacetate, as well as hexose monophosphate shunt metabolites, ribose-5-phosphate and sedoheptulose 7-phosphate were significantly increased in fibroblasts isolated from IPF patients relative to cells from control subjects. Cofactor nucleotides including ATP and NADH were significantly decreased, while FAD, ADP, AMP, NADP, NADPH were increased significantly in IPF lung fibroblasts. Further studies with a HIF-1 prolyl hydroxylase (PHD) competitive inhibitor, dimethyloxaloylglycine (DMOG) or cofactor, α-KG in vitro revealed the importance of this TCA metabolite in regulation of myofibroblast differentiation by modulation of HIF-1 stability/degradation. Thus, metabolic regulation of myofibroblast differentiation during the pathogenesis of idiopathic pulmonary fibrosis possibly mediated by HIF-1 is suggested.

oral 011	15 min	NIH cancer * lung diseases	Nicholas Kenyon

Profiling of downstream metabolites of L-arginine in severe asthmatics

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Introduction: Patients with severe asthma do not respond well to standard therapies of inhaled corticosteroids and bronchodilators. Nutritional interventions, such as L-arginine supplementation, may provide a safe and inexpensive alternative treatment strategy for severe asthmatics. This pilot grant addresses the need to identify metabolic profiles derived from L-arginine supplementation. These profiles may be used to pinpoint cohorts of severe asthmatics that respond more favorably to the intervention by determining whether changes in L-arginine metabolites measured in peripheral blood correlates with responses to L-arginine. The study is a double-blind, placebo-controlled, cross-over intervention trial. Severe asthmatics subjects are recruited from a referral-based specialty clinic and randomized to a low (25ppb) exhaled nitric oxide group. All subjects receive L-arginine (0.05g/kg twice daily) and placebo for three months with a two month washout period. Serum iss collected 0, 6 and 12 weeks. Serum was extracted for GC-TOFMS and HILIC-QTOFMS analysis. For GC-TOF, samples were derivatized and analyzed using Pegasus IV and raw data deconvoluted using ChromaTOF. HILIC-QTOFMS samples were analyzed using Agilent 6530 QTOFMS in ESI (+) mode and raw data processed using MSDial for peak finding and identifications. MSMS matching and mz/RT in house library were used for identifications. Results: GC-TOFMS and HILIC-TOFMS platforms provided in depth coverage of primary and polar secondary L-arginine metabolites. Both platforms were used to interrogate the differences in arginine metabolism levels in the patient samples and yielded excess of 300 annotated metabolites. At the time of abstract submission, investigators remain blinded to subject grouping. We created correlation maps showing the relationships of arginine to selected products resulting from L-arginine metabolic pathways, including ADMA and SDMA, ornithine, and citrulline. Spearman rank correlations indicate a strong relationship between the concentration of serum L-arginine and ornithine. Both ornithine and citrulline show a strong positive correlation to serum L-arginine concentration (ornithine vs. arginine $p<0.001$, citrulline vs. arginine $p<0.001$). Interestingly, L-arginine also positively correlated with combined ADMA/SDMA (ADMA/SDMA vs. arginine $p<0.001$).Correlation of metabolites with clinical outcomes will be necessary to determine severe asthma responders to L-arginine. ADMA/SDMA levels are serum markers that will ultimately determine the safety profile of this intervention. L-arginine concentrations strongly correlate with concentrations of both ornithine and citrulline signifying that both the arginase and nitric oxide synthase pathways are upregulated systemically. HILIC-TOFMS can be used to measure downstream metabolites of L-arginine that profile critical pathways in severe asthma.

oral 012	15 min	NIH CNS * other diseases	Eugenia Trushina

Metabolic Alterations in Patients with Alzheimer's Disease.

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Alzheimer's Disease (AD) currently affects more than 5 million Americans, with numbers expected to grow. The pathophysiological changes in AD patients begin decades before the onset of dementia, highlighting the urgent need for the development of early diagnostic methods. Using non-targeted and targeted metabolomic approaches, we reported presence of signatures of early energetic stress and mitochondrial dysfunction in the brain tissue of multiple transgenic animal models of AD, and plasma and cerebrospinal fluid of patients with AD. To determine the exact mechanism of alterations, we utilized stable isotope tracers ([U-13C]-glucose and [U-13C,15N]-glutamine) and mass spectrometry to measured the TCA flux in primary fibroblasts from male and female patients of AD. Using stable isotope tracers and we examined alterations in glucose metabolism in primary human fibroblasts from patients with sporadic AD and age- and sex-matched control individuals. Using [U-13C]-glucose as a tracer, we determined the extent of both glucose consumption and lactate production, and metabolism through the TCA cycle. Using [U-13C,15N]-glutamine as a tracer, we examined glutamine import and metabolism through the TCA cycle in AD patient fibroblasts relative to control cells. These data provide the insights in whether glutamine is utilized for an alternative glucose- independent TCA cycle. Data will be compared to our previous results obtained in the CSF and plasma from MCI and AD patients. Based on the changes identified in the TCA cycle in AD compared to control fibroblasts using stable isotope-labeled glucose and glutamine tracers, we expect to identify the relationship between glucose-dependent and -independent TCA cycle activity. We will discuss to what extent different patients with sporadic AD share similar alterations in the TCA cycle, and to whether these changes are consistent with changes in metabolites identified in the CSF and plasma in our previous work. We will also discuss to what extent metabolic signatures in control and AD subjects differ with sex. Identification of the enzymatic pathways affected in AD could provide a foundation for therapeutic interventions. Comparison of metabolic alterations in peripheral cells, CSF and plasma Sex effect on metabolic signatures in AD and healthy individuals

oral 013	15 min	NIH CNS * other diseases	Pei-an Betty Shih

The Soluble Epoxide Hydrolase "Omics-Trio" Revealed Potential Treatment Targets for Anorexia Nervosa

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Anorexia nervosa (AN) is characterized by severe restrictive eating and emaciation, with high rates of morbidity, chronicity and mortality. Current treatments are ineffective and have a high rate of relapse, thus developing new treatments is a high priority in mental and public health. A limiting factor in developing new effective treatments is a lack of adequate knowledge on how molecular mechanisms of disease genes affect pathophysiology. We have taken an integrative multi-domain Omics (genomic, proteomic, lipidomic and metabolomics) research approach to investigate how molecular mechanisms of an AN susceptibility gene, soluble Epoxide Hydrolase 2 (EPHX2), that was discovered through our GWAS and exon sequencing studies, effects AN. Lipidomic and metabolomic assays were

conducted using the GC/MS and LC/MS/MS systems. Quantitative profiling of polyunsaturated fatty acids (PUFAs), the parental substrates of soluble epoxide hydrolase (sEH), was done using plasma of 60 female AN patients and 36 healthy control women. Targeted oxylipin profiling was performed in 20 patients and 38 controls. Additional fasting and postprandial oxylipins were obtained in 6 patients and 5 controls to assess intraindividual oxylipin shift. Ex vivo sEH activity was directly measured in the buffy coats of 15 patients and 5 controls. Diol:epoxide oxylipin ratios and ω6:ω3 PUFA ratios were calculated as proxy markers of in vivo sEH activity and dietary PUFA markers, respectively. All statistical analyses were performed in R.3.1.3. Both the short-chain (LA:ALA) and long-chain (ARA:EPA) ω6 to ω3 PUFA ratios were lower in patients compared to controls (127 versus 196, $p<0.001$ for LA:ALA and 8 versus 17, $p<0.0001$ for ARA:EPA). Cytochrome P450 metabolites of PUFAs were associated with AN and showed a pattern of higher diol:epoxide oxylipin ratios in AN patients, suggesting that in vivo sEH activity is elevated in patients compared to controls. The direct ex vivo sEH activity measurement demonstrated higher activity in patients compared to controls (0.012 versus 0.007 nmol.min-1.mg-1, $p=0.05$), which is consistent with the data of sEH activity proxy markers. PUFAs are upstream parental substrates of sEH, therefore the dysregulation of PUFA profile observed in patients may play a role in the sEH-associated AN risk. To account for substrate-mediated effect and to explore metabolic consequences of sEH activity in AN, postprandial oxylipins that are substrates (epoxy-fatty acids) of sEH and the resulting metabolites (diol-fatty acids) were assessed and compared in patients and controls. After accounting for the potential confounding effects of pre-catalyst epoxy-fatty acid level (5.6.EET) and age, the postprandial shift of pro-inflammatory diol-fatty acid of ω-6 ARA, 5.6.DiHET, was more than 3-times higher the level observed in controls (1.51 versus -4.09, $p=0.09$). Interestingly, the diol-fatty acids from ω-3 ALA and DHA did not show significant difference between groups. The use of multi-domain EPHX2 omics markers reveals that upregulated sEH activity and resulting pro-inflammatory shift may be the key mechanism by which EPHX2 effects AN risk. Furthermore, dietary factor may modulate the deleterious effect sEH plays on AN risk, illness course, and outcome. This study is timely because both enzyme inhibition and dietary modulation are accessible approaches for AN treatment development.

oral 014	15 min	NIH CNS * other diseases	Jaspreet Singh

Untargeted metabolomics to reveal genotype-phenotype correlation in X-linked adrenoleukodystrophy

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Phenotype variability in inherited neurometabolic disease X-linked adrenoleukodystrophy (X-ALD) can be a matter of life and death ranging from benign adrenomyeloneuropathy (AMN) to severe fatal cerebral adrenoleukodystrophy (ALD). Primary gene defect (mutation/deletion in peroxisomal ABCD1) and the resulting accumulation of saturated very long chain fatty acids (VLCFA; C>22:0) in tissues (including brain) is common to all the phenotypes of X-ALD disease and hence, cannot predict the phenotype and disease course. We recently reported the loss of metabolic enzyme AMP-activated protein kinase (AMPK) in ALD patient-derived fibroblasts but not in AMN. AMPK is the “master regulator” of cellular metabolism and raises the exciting possibility that metabolic pathways and their products may be uniquely altered between AMN and ALD patients. Fibroblasts derived from healthy controls (CTL) and subjects with mild (AMN) and severe (ALD) forms of disease were cultured in DMEM with 15% FBS and antibiotic (pen/strep). After 24h cultures were supplemented with fresh media for 3hr. Cells (2x106) were snap frozen and stored at -80c until processed. Metabolites were extracted using Methanol:Chloroform:Water (8:1:1), reconstituted in methanol:H2O (1:1) and processed using Accurate-Mass Q-TOF LC/MS. 1290 Infinity Binary LC System (Agilent Technologies) was used for chromatographic separation and 6530 Accurate-Mass Q-TOF (Agilent Technologies) with a dual ASJ ESI ion source was used as the mass detector. Raw data processing was done using Agilent software (MassHunter Qual and ProFinder). Data analysis was performed in R (http://cran.r-project.org/) and by “MetaboAnalyst 2.5” (http://www.metaboanalyst.ca/). We profiled the global metabolome using liquid chromatography coupled with mass spectrometry to identify the relative levels of metabolites in patient-derived fibroblasts from healthy control (CTL, N=6), mild (AMN, N=6) and severe (ALD, N=6) disease. Quality of analysis was accessed by visual inspection of the chromatographic traces (total ion chromatograms) and relative quantification of the internal standards. Quality control data suggest that the analysis method was stable and reproducible across all samples. There were 2972 peaks identified from the LC/MS+ spectra with 1587 detected in at least 75% of the samples. PLS-DA revealed a clear separation between CTL, AMN and ALD groups, indicating presence of unique

metabolite profiles for the CTL, AMN and ALD groups. Analysis of variance per metabolite identified 194 biochemical peaks that differ between the three groups ($P<0.05$; FDR<0.35). Post-hoc t-tests determined that 97 of these were differentially altered between AMN and ALD patient-derived fibroblasts, indicating altered metabolomics profile in AMN and ALD phenotypes. Among these 97 altered biochemical peaks, 33 were mapped to specific metabolites. Pathway analysis in Metaboanalyst identified 5 pathways that were significantly enriched in this set of 33. These included glycerophospholipid metabolism ($P<0.00371$), cysteine and methionine metabolism ($P<0.0103$), pantothenate and CoA biosynthesis ($P<0.0203$), beta-Alanine metabolism ($P<0.0218$), glutathione metabolism ($P<0.0386$) and galactose metabolism ($P<0.0443$). Initial analysis, presented here, focuses on positive ion separation (LC/MS+). Future integration of the LC/MS- data, also available, will further enhance our understanding of the metabolic alterations across the spectrum of disease from Healthy to AMN to ALD. Gene defect and VLCFA accumulation are similar in X-ALD phenotypes. Metabolic alterations may serve as biomarkers and disease-modifying therapeutic targets.

oral 015	15 min	NIH CNS * other diseases	Laura Cox * Early Career Scientist

Systemic metabolic impact of early-life microbiota disruption

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The early-life microbiota plays a role in shaping host growth and development. Microbiome disruption via antibiotics early in life (eg. during delivery by Caesarian section or treatment of infections) increases the risk of overweight status later in childhood. Low dose antibiotics have been shown to increase weight gain and adiposity in a wide variety of animal species and these changes can be mediated by the microbiota alone, as demonstrated by microbiota transfer to germ-free mice. Furthermore, disruption only during infancy is sufficient for lasting metabolic changes. In a model of antibiotic- and microbiota-induced obesity, we sought to determine how endogenous metabolism is linked to host-transcriptional changes, microbial composition, and metabolic potential. We administered low-dose penicillin (LDP) to conventional SPF mice, and measured changes in body composition by dual energy x-ray absorptiometry and MRI. To examine causal relationships, we transferred cecal microbiota collected from control or LDP mice into germ-free recipients. We used NMR metabolomics to analyze metabolites from liver and cecal contents from female mice at 8 weeks of age in both models. Statistical and multivariate methods were used to determine bins that differentiated the study phenotypes, and metabolites were matched used a library of metabolite standards. Hepatic transcription was surveyed by RNAseq, and microbiota composition and potential function were characterized by high-throughput sequencing and PICRUSt. Conventional SPF mice receiving LDP or germ-free mice colonized with LDP-microbiota had greater total and fat mass compared to their respective controls. That specific microbial taxa were consistently suppressed during infancy in multiple independent experiments suggests that these taxa may have beneficial roles in metabolic development. PICRUSt analysis indicated reduced carbohydrate and elevated lipid and protein microbial metabolic pathways in LDP mice or LDP-microbiota recipients relative to the control mice. Mice receiving either LDP or LDP-microbiota had differences in the cecal and hepatic metabotypes compared with the control mice, and four differentiating metabolites were shared between both models: cecal ethanol, hepatic glutathione, maltose, and acetate. Cecal ethanol was elevated, indicating that microbiota disruption altered metabolic end-products from carbohydrate fermentation. Hepatic glutathione was significantly decreased, while the expression of hepatic glutathione S-transferase was significantly elevated. These results provide evidence that there was enhanced oxidative stress in the LDP mice. Hepatic maltose was elevated, and may accumulate due to reduced microbial carbohydrate metabolism. Finally, hepatic acetate was elevated which may contribute to metabolic signaling. These studies characterize important variables in early-life microbe-host metabolic interactions, identifying several metabolites consistently linked with morphometric alterations.

oral 016	15 min	NIH CNS * other diseases	Ellen Weiss

Metabolomic Profiling of Early Events in Retinal Degeneration

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The vertebrate retina has one of the highest metabolic activities of any tissue in the body due to a requirement for energy that changes continuously in response to varying light conditions. Therefore, oxidative stress appears to be a contributing factor in many retinopathic conditions caused by environmental and genetic insults. However the metabolic dysfunctions that trigger retinal degeneration are unknown. Using wild type mice and an established genetic model for retinal degeneration, the rd10 mouse, we have employed metabolomics to identify the key metabolites that contribute to retinal degeneration. Since degeneration is accelerated in rd10 mice by light, we have also compared metabolic profiles of wild type and rd10 mice raised in normal cyclic light and in complete darkness. Wild type C57Bl6/J and rd10 mice raised in cyclic light or in the dark were euthanized on postnatal day 18. After euthanasia, retinas were removed, frozen in liquid nitrogen and stored at -80 ˚C prior to analysis. Frozen retinas were solubilized in buffer containing 50:50 acetonitrile:water. The extracts were separated using centrifugation, lyophilized, re-constituted in 95:5 H2O:methanol and analyzed by Ultra Performance Liquid Chromatography-Time-of-Flight-Mass Spectrometry (UPLC-TOF-MS). The data were processed using Waters' Progenesis QI software and subjected to statistical and multivariate analysis (e.g., PCA, OPLS-DA) using the SIMCA (Umetrics) software to determine the metabolites that best separate the groups based on inspection of loadings and VIP plots. Data were collected over 50-1000 m/z in both positive and negative ion electrospray modes. Approximately 3,000 compound ions were detected in these samples. About 700 compounds were matched with compounds in metabolome databases. Principal Component Analysis (PCA) demonstrated that metabolomic profiles of rd10 mice raised in cyclic light cluster separately from rd10 mice raised in the dark, indicating that light rearing induces major changes in the metabolite profile. In contrast, there were fewer differences between light-reared and dark-reared wild type mice suggesting an enhanced sensitivity of the rd10 mice to light. Orthogonal Projections to Latent Structures-Discriminant Analysis (OPLS-DA) was used to determine differences between the genotypes as well as the effect of light conditions during rearing. Pairwise comparisons indicated that rearing mice in the light results in different metabolic profiles for both rd10 and wild type mice compared to rearing them in the dark. There were also differences in metabolic profiles between rd10 and wild type mice raised under the same light conditions. These results, using an unbiased broad-spectrum analysis, demonstrate for the first time that retinas from rd10 and wild type mice have distinct metabolic profiles when raised under either light or dark conditions. Variable importance plots (VIP), generated through OPLS-DA analysis, were used to determine the group differentiating metabolites (those with a VIP>1). Many classes of compounds were noted to have a VIP>1, including those that participate in mitochondrial function. Interestingly, there were also a large number of unknown metabolites with a VIP>2. These compounds may represent novel metabolites or metabolites that are not in the Human Metabolome Database, indicating the existence of a large number of unknown metabolites in the retina that contribute to retinal degeneration. Future studies will focus on identifying these critical unknown compounds as well as the relevant metabolic pathways. These studies demonstrate the power of metabolomics as a novel approach for identifying critical factors that trigger retinal degeneration.

oral 017	15 min	NIH CNS * other diseases	Brittany Lee-McMullen * PhD Student

Metabolomic Analysis of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a fatal muscle degenerating disease caused by the absence of the dystrophin protein. Replacement of healthy muscle by lipid and fibrotic tissue results in boys with DMD succumbing to the disease in the third decade of life. Promising clinical trials are impeded, as current outcome measures lack therapeutic sensitivity and are susceptible to inherent limitations. As a result, there is need for sensitive and non-invasive biomarkers to track disease progression and prognosis in boys with DMD. In this study, we investigate the urine from boys with DMD using both NMR and MS. Metabolomics findings were then analyzed against a database of phenotype and genotype data for each boy including MRI, functional assessments, and medication logs. Urine was collected from boys with (n=54) and without (n=7) DMD ranging in age from 5–17. All metabolomics data was acquired by the Southeast Center for Integrated Metabolomics (SECIM), following protocols as defined on the Metabolomics Workbench. Urine 1D NOESY and 2D JRES, HSQC, and TOCSY spectra were acquired using a 600 MHz Bruker NMR spectrometer with cryogenic probe. Spectra were analyzed using in-house MATLAB scripts for quality control and analysis workflows. Targeted mass spectrometry was performed at Sanford Burnham. Both organic acids and amino acids were determined. Creatinine was quantified independently for each samples using UV detection. A database of phenotypic and genomic data was collected and maintained as part of the ImagaingDMD natural history trial. The major findings in this study were that the ratio of creatine to creatinine concentrations track with disease progression. In control urine, creatinine levels were 1.5 fold higher ($p=0.001$), with next to no creatine present ($p<0.05$). However, in the urine of boys with DMD, the Creatine/Creatinine levels were 4 fold higher that of control ($p=0.01$). These finding suggest an alternation of the creatine and creatinine regulation in DMD boys compared to controls. We then used the creatine/creatinine ratio to correlate to the MRI T2 measurements (index of disease progession). MRI T2 increases in damaged tissue compared to healthy tissue. We discovered that as the T2 increased, the creatine/creatinine ratio increased as well ($R2 = 0.244$, $p=0.0001$). This gives insight into the state of the tissue, providing a diagnostic window into DMD. We then determined if the creatine/creatinine ratio changed with disease progression. Of the 18 longitudinal measurements (0.8 years ± 0.25 years), only in 10 creatine/creatinine increased, all of which had been on a consistent medication regimen. Although eight decreased, 3 had recently started on therapeutic levels of steroids and 3 others were on experimental medication regimens. These promising results show that the urine metabolic profile can be used to track disease progression in DMD and is sensitive to therapeutic changes. We are currently evaluating the data for additional metabolomic biomarkers of DMD disease progression. We correlated DMD metabolomics to a database of MRI, functional and genomic data,to track DMD progression.

oral 018	15 min	NIH metabolic syndrome * kidney	Rhonda Cooper-DeHoff

Pharmacometabolomics identifies acylcarnitines associated with beta blocker induced fasting glucose alterations

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B-blockers, commonly prescribed for hypertension treatment, are proven blood pressure lowering agents. However they are also associated with adverse metabolic effects, including hyperglycemia and diabetes. Knowledge regarding the mechanistic underpinnings of β-blocker-associated dysglycemia is incomplete. Identifying a biomarker that predicts the onset of dysglycemia accompanying b-blocker therapy could lead to a more personalized and safer

approach for hypertension management. Acylcarnitines (ACs) are a large class of metabolites (derived from the corresponding acyl-CoAs) that signal the involvement of a host of different metabolic pathways. We used a targeted pharmacometabolomics approach focused on ACs to probe for a possible association between the baseline AC signature and dysglycemia after treatment with the commonly prescribed β-blocker, atenolol. Caucasian hypertensive patients (n=225) who were treated with atenolol in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study are included in this study. A targeted LC/MS assay was utilized for the quantitative measurement of 57 ACs in baseline serum. 23 ACs (no or low quantification) and 1 patient (outlier values) were excluded. For the ACs detected in 90% of all patients (n=29), we conducted linear regression for change in glucose (difference between baseline and end of atenolol) with baseline AC level, baseline glucose, age, sex, and BMI as covariates. For ACs detected in at least 50% of the patients (n=5), we modeled change in glucose as described, but with AC included as present/absent in the regression. Patients were 49.7±9.4 years old, 48% female, with mean body mass index of 30.2±5.5 kg/m2, mean blood pressure 145/93 ± 9.4/5.6 mm Hg, mean baseline glucose of 91.4±10.3 mg/dl and mean glucose change of 2.2±9.3 mg/dl. We identified a cluster of long-chain ACs that were associated with glucose change following atenolol treatment. Presence (vs absence) of arachidonoyl-carnitine (C20:4), p=0.0007, as well as increasing concentrations of stearoyl-carnitine (C18), p=0.04 and palmitoyl-carnitine (C:16), p=0.04 was significantly associated with atenolol-induced hyperglycemia. Additionally oleyl-carnitine (C18:1), p=0.05 and linoleoyl-carnitine (C18:2), p=0.07 trended towards significance. Previous reports have identified the association of long-chain ACs with obesity, insulin resistance and diabetes. Additionally, there is evidence from our prior work that the oleic acid, palmitic acid and linoleic acid levels are significantly associated with increased blood glucose after atenolol treatment, and data from the literature demonstrate that arachidonic acid is strongly associated with glucose regulation. Hence, our findings in this RCMRC pilot study, the first to our knowledge, to associate ACs with drug induced hyperglycemia, are biologically plausible and worthy of further investigation and confirmation. Precision medicine and data-driven prescribing is quickly becoming a reality due to revolutionary advances, especially pertaining to “omics platforms”. Application of pharmacometabolomics to classify individuals into subpopulations that differ in their response to a specific treatment is an important enabler of precision medicine. The data from our pilot study suggest that the AC signature in blood serum from patients treated with a β-blocker may yield an informative biomarker that can be leveraged to devise a personalized approach for the treatment of hypertension. Pharmacometabolomics, a tool that identifies signatures related to drug response or adverse effects, can inform the goal of precision medicine.

oral 019	15 min	NIH metabolic syndrome * kidney	Olivia Osborn

Metabolite Changes Associated with Obesity and Weight Loss

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A chronic imbalance between energy intake and energy expenditure results in excess fat deposition and the development of obesity. Obesity induces changes at the gene expression, protein and metabolite levels in peripheral tissues as well as the central nervous system that drive the development of associated co-morbidities including insulin resistance, diabetes and cardiovascular disease. In these studies we investigated the metabolic changes induced in mice by obesity after chronic high fat diet feeding and after weight loss. We performed both untargeted (complex lipids) and targeted (Endocannabinoids/ oxylipins/ceramide) metabolomic analysis of insulin target tissues from lean, obese and lean mice that were previously obese. Male C57BL6 mice were divided into three groups. One group was fed a high fat (HF) diet (60% calories from fat, Research Diets) for 18 weeks to induce obesity. A lean group was fed a low fat (LF) diet (10% calories from fat, Research diets) for 18 weeks. A third group was fed the HF diet for 9 weeks and then switched (SW) to the LF diet for a further 9 weeks to induce weight loss. At the end of the study mice were sacrificed and tissues (liver, adipose, muscle, hypothalamus and plasma) collected for metabolomic studies. We have identified changes in metabolites that occur in the key insulin target tissues, including adipose, liver, muscle, hypothalamus in the obese state that are reversed upon weight loss. These reversible metabolites are likely associated with insulin resistance in the obese state and insulin sensitivity in the lean state. Furthermore, we have identified metabolites that are induced by obesity that do not reverse upon weight loss. We hypothesize that metabolites that are irreversibly changed in obesity may play a role in driving weight regain and may provide

important targets for therapeutic intervention in maintenance of weight loss. Determination of the extent to which weight loss reverses the metabolomic changes induced by obesity.

oral 020	15 min	NIH metabolic syndrome * kidney	Charles Evans

Metabolomics and fluxomics in vivo: applications to study of exercise

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Metabolomics is best-suited among the 'omics sciences to assess changes associated with biological events that take place over short timeframes, such as response to a meal or aerobic exercise. To obtain a complete picture of such events, measurements of both metabolite levels and metabolic flux are desirable. In "simple" systems such as microorganisms or cultured cells, this can be accomplished by performing metabolomics with and without the use of stable isotope tracers. In mammals, it is much more challenging to assess flux given the environment of multiple organs with complex interactions. Yet the challenge is worth confronting, since metabolic response to short-term phenomena such as exercise has been shown to influence risk for major health concerns including diabetes and obesity. We describe the use of stable isotope-resolved metabolomics to assess flux in skeletal muscle and other organs. Sprague-Dawley rats and several other strains were injected intraperitoneally with 13C glucose, 13C octanoic acid, or 13C15N valine, and were exercised on a treadmill or rested. Tissues including skeletal muscle, liver, and adipose were carefully harvested and analyzed by liquid chromatography – mass spectrometry (LC-MS) to measure metabolite levels and incorporation of 13C into metabolic pathways including glycolysis, the TCA cycle, and lipid metabolism. Our data reveal patterns of alterations in both metabolite levels and metabolic flux in response to exercise. 45% 13C enrichment (sum of all isotopes) was observed in citric acid from gastrocnemius muscle of exercised rats, compared to 20% in rested animals. The levels of some metabolites, including certain TCA cycle intermediates and acylcarnitines, differed between animals with higher and lower exercise capacity. However, the use of 13C glucose as a tracer during exercise did not reveal apparent differences in metabolic flux in skeletal muscle of these animals. On the other hand, 13C octanoic acid and 13C valine showed evidence of elevated levels of lipid and branched chain amino acid metabolism in skeletal muscle of rats with high exercise capacity. Our study demonstrates the potential of isotope-resolved metabolomics to probe metabolism in vivo and highlights the potential advantages and remaining challenges associated with its use to study complex biological phenomena and major diseases. Isotope-resolved metabolomics was used to assess flux in mammalian tissues during exercise and its benefits and challenges are discussed.

oral 021	15 min	NIH metabolic syndrome * kidney	Brian O'Neill

Impact of Muscle Insulin and IGF-1 Signaling on Serum and Muscle Metabolite Profiles

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Skeletal muscle insulin resistance is a cardinal feature of the pathogenesis of type 2 diabetes. Insulin resistance is strongly associated with altered mitochondrial function, but whether one causes the other is debated. Altered plasma amino acid (AA) metabolites, including increased branched chain amino acids (BCAAs), occurs in insulin resistant and deficient states and are reported to predict type 2 diabetes in people. Indeed, insulin deficiency in humans causes muscle protein degradation and increased efflux of amino acids into the systemic circulation. However, it remains to be determined whether changes in muscle mitochondrial function or circulating amino acid metabolites occur due to direct impact of reduced insulin action on muscle or secondary to other systemic changes related to the pre-diabetic state. We generated mice with muscle-specific deletion of both insulin receptors (IR) and IGF-1 receptors (IGF1R) using Cre lox recombination. These MIGIRKO mice do not develop diabetes, but do display marked muscle atrophy. To test how loss of insulin/IGF-1 signaling in muscle perturbs amino acid and mitochondrial metabolites, we performed quantitative metabolite profiling on serum and muscle from MIGIRKO mice as well as mice lacking IR or

IGF1R alone in muscle, and compared these changes to insulin resistant diet-induced obese (DIO) mice. We correlated changes in metabolites with analyses of mitochondrial functional as well as measures of protein turnover within muscle. Tricarboxylic acid (TCA) cycle metabolite profiling in muscle from MIGIRKO mice showed accumulation of glutamate, fumarate, and citrate; changes that were not present in DIO mice or mice lacking IR or IGF1R alone. These TCA metabolite changes in MIGIRKO muscle agree with observed decreases of mitochondrial respiration in isolated mitochondria using glutamate/malate as substrates, a phenomenon that was not observed with succinate as a substrate. These data demonstrate that loss of IR/IGF1R signaling impairs mitochondrial respiration through complex I leading to accumulation of glutamate and TCA cycle metabolites. Serum amino acid metabolites from of MIGIRKO mice showed minimal changes, without any changes in BCAAs. However, amino acid metabolite profiles from MIGIRKO muscle tissue display increases in BCAAs and many other AA metabolites that are typically altered with insulin deficiency. Elevations in BCAAs also occurred in muscle tissue from DIO mice compared to chow diet controls. Muscle from MIGIRKO also showed marked decreases in histidine metabolites and changes in glutamate and aspartate metabolites, which were associated with increases in markers of protein degradation via autophagy–lysosome. To determine if inhibition of autophagy could change amino acid metabolites in muscle from MIGIRKO or DIO mice, we treated these and control mice with colchicine, a known inhibitor of autophagy in muscle. While autophagy inhibition had minimal effects on metabolites from MIGIRKO muscle, DIO mice showed normalization of muscle BCAA levels with colchicine treatment. Thus, disruption of insulin/IGF-1 signaling in muscle induces mitochondrial dysfunction and alters BCAA/other AA metabolites in part via autophagy-lysosomal degradation.

oral 022	15 min	NIH metabolic syndrome * kidney	Snezana Petrovic

Urine Metabolomics Profile in Early CKD

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Urine is an easily obtained biological fluid that is useful for metabolomics analysis and holds promise for uncovering metabolic differences between normal and disease states, including chronic kidney disease (CKD). A conspicuous gap remains between the low sensitivity and retrospective nature of current clinical markers, our inability to predict those who will progress to end-stage kidney disease (ESRD), and the high prevalence of CKD in the U.S. (~26 million individuals). Patients with CKD typically have significant co-morbidities and high rates of cardiovascular mortality. Metabolomics may be informative to detect and intervene at early stages of CKD with great benefit to public health. Urinary metabolomics profiles of 30 African American-Diabetes Heart Study participants with Stages 2 and 3 CKD (eGFR=71.7±28.4ml/min/1.73m2; urine albumin:creatinine ratio [UACR]=478.5±774.2 mg/g; age=56.6±9.5 years; BMI=39.1±10.0 kg/m2) and 47 diabetic non-nephropathy controls (eGFR= 91.3 ± 20.4ml/min/1.73m2; UACR= 8.2 ± 6.4 mg/g; age=53.8±8.0 years; BMI=37.3±8.9 kg/m2) were compared. Data were generated using broad spectrum 1H NMR spectroscopy; the raw data binned and normalized to total integral of each spectrum to account for differences in urine concentration. Binned data were mean centered, pareto-scaled, and subjected to multivariate analysis, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Bins important for distinguishing cases and controls were identified using variable importance to projections (VIP) plots (VIP≥1) and library matched for metabolites. A specific set of metabolites differentiating diabetic individuals with CKD from those without CKD was identified: 1-methylhistidine, 3-methylhistidine, citrate, creatinine, dimethylamine, glutamate, glutamine, glycylproline, leucine, myo-inositol, N-phenylacetylglycine, o-phosphoserine, phenylalanine, proline, S-sulfocysteine, salicylurate, succinylacetone, and valine. All African American-Diabetes Heart Study participants have diabetes; cases and controls with long diabetes durations (≥14 years) were analyzed as a separate subgroup because diabetes duration is important in adjudicating diabetic nephropathy. The set of metabolites that differentiated cases from controls in this subgroup contained some previously identified metabolites (citrate, creatinine, dimethylamine, and valine) in addition to 2-methylglutarate,

arginine, guanosine triphosphate, lysine, methylsuccinate, myo-inositol, N-acetyltyrosine, putrescene, and salicylurate. A subgroup of 40 patients was followed longitudinally for 3-5 years, and we were able to identify a distinct set of metabolites differentiating those patients who maintained stable kidney function (rate of eGFR decline<1ml/min/1.73m2/year) compared to individuals whose rate of eGFR declined >1ml/min/1.73m2/year: 2-oxoisocaproate, 3-hydroxybutyrate, 3-methyl-2-oxovalerate, 3-methylhistidine, formate, isoleucine, leucine, maltose, and methylsuccinate. Our study therefore defined three sets of urinary metabolites that associated distinctly with early CKD, diabetic CKD, and CKD progression. A number of these metabolites were amino acids (AA) and their derivatives, and altered serum AA profile has previously been associated with severity of non-diabetic kidney disease. Alterations in protein homeostasis are characteristic of advanced CKD, but it appears that alterations in AA homeostasis can be detected in early stages of CKD. Several metabolites imply changes in tricarboxylic acid cycle, consistent with the reported mitochondrial dysfunction in diabetic nephropathy. This study suggests that metabolomics may improve the detection of early kidney dysfunction from diabetes and may predict nephropathy progression.

oral 023	15 min	NIH metabolic syndrome * kidney	Patrick Brophy

Metabolomics profiling of renal development and acute kidney Injury in premature infants

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Neonatology represents an at- risk population that little is understood in the development and impact of Acute Kidney Injury (AKI). Standard codification of stages of AKI have been developed and validated in both pediatric and adult populations yet these criteria do not exist for the neonatal population. Creatinine as a measure of renal dysfunction suffers from considerable confounders. Given the paucity of data available for neonatal patients in terms of alternative biomarkers for AKI, we developed a metabolomics based strategy to define a normative (no AKI) data set for pre-term infants across gestational age groups and to identify differences in urinary metabolic profile in preterm infants with AKI based on commonly used birth weight and gestational age indices. Urinary metabolomic profiles in preterm newborn infants (≤ 31 weeks) with “no AKI” were compared to matched infants that had developed stage 1 and stage 2/3 AKI as defined by modified 2012 KDIGO AKI definition. A total of 40 urine samples were analyzed on the 2nd postnatal day using NMR-based metabolomics analysis with as phenotypic anchors and stratified by clinical diagnosis of AKI. Multivariate data analysis (PCA, OPLS-DA) was conducted. Variable importance to projections (VIP) plots were used for identifying bins deemed to be important for separation of the study groups (VIP≥ 1.0). A specific set of metabolites differentiating diabetic individuals with CKD from those without CKD was identified: 1-methylhistidine, 3-methylhistidine, citrate, creatinine, dimethylamine, glutamate, glutamine, glycylproline, leucine, myo-inositol, N-phenylacetylglycine, o-phosphoserine, phenylalanine, proline, S-sulfocysteine, salicylurate, succinylacetone, and valine. All African American-Diabetes Heart Study participants have diabetes; cases and controls with long diabetes durations (≥14 years) were analyzed as a separate subgroup because diabetes duration is important in adjudicating diabetic nephropathy. The set of metabolites that differentiated cases from controls in this subgroup contained some previously identified metabolites (citrate, creatinine, dimethylamine, and valine) in addition to 2-methylglutarate, arginine, guanosine triphosphate, lysine, methylsuccinate, myo-inositol, N-acetyltyrosine, putrescene, and salicylurate. A subgroup of 40 patients was followed longitudinally for 3-5 years, and we were able to identify a distinct set of metabolites differentiating those patients who maintained stable kidney function (rate of eGFR decline<1ml/min/1.73m2/year) compared to individuals whose rate of eGFR declined >1ml/min/1.73m2/year: 2-oxoisocaproate, 3-hydroxybutyrate, 3-methyl-2-oxovalerate, 3-methylhistidine, formate, isoleucine, leucine, maltose, and methylsuccinate. Our study therefore defined three sets of urinary metabolites that associated distinctly with early CKD, diabetic CKD, and CKD progression. A number of these metabolites were amino acids (AA) and their derivatives, and altered serum AA profile has previously been associated with severity of non-diabetic kidney disease. Alterations in protein homeostasis are characteristic of advanced CKD, but it appears that alterations in AA homeostasis can be detected in early stages of CKD. Several metabolites imply changes in

tricarboxylic acid cycle, consistent with the reported mitochondrial dysfunction in diabetic nephropathy. This study suggests that metabolomics may improve the detection of early kidney dysfunction from diabetes and may predict nephropathy progression.

oral 024	30 min	lung and cardiovascular diseases	Stanley Hazen

Therapeutic approaches targeting gut microbial contribution to cardiometabolic disease

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Untargeted metabolomics studies first discovered the potential involvement of a specific gut microbial pathway and heart disease risk. Specifically, ingestion of dietary nutrients with trimethylamine (TMA) moiety, such as from phosphatidylcholine, choline or carnitine, leads to generation of TMA in a gut microbe dependent fashion, followed by host hepatic flavin monooxygenase (FMO) 3 dependent conversion into the product trimethylamine-N-oxide (TMAO). Plasma levels of TMAO are associated with cardiovascular disease risks, and provision of either TMAO directly, or nutrient precursors in the presence of gut microbiota (and TMAO formation) accelerates atherosclerosis in animal models. Leveraging this knowledge for development of therapeutic interventions to prevent cardiovascular disease has not yet been reported. Discovery of the link between the TMAO metaorganismal (involving both microbe and host) pathway and atherosclerotic heart disease has led to several potential approaches toward preventing formation of TMAO, and testing whether this will impact atherosclerosis development in vivo. Therapeutic approaches to be discussed include: (i) dietary intervention studies aimed at reducing TMAO levels; (ii) targeting host FMO3 inhibition and its impact on diet induced atherosclerosis; (iii) non-lethal microbial enzyme inhibition and its impact on TMA and TMAO production in vivo, and atherosclerosis. Human studies will be discussed examining the impact of both chronic dietary exposure to choline or carnitine and plasma TMAO levels in subjects, as well as specific dietary efforts designed to inhibit TMA formation and suppress plasma TMAO levels in subjects. In addition, the effect of genetically targeting FMO3 on animal models of atherosclerosis will be discussed. Finally, the impact of targeted inhibition of the first step in TMAO generation, microbial TMA lyases, on diet-induced atherosclerosis will be discussed. Dietary, pharmacological or probiotic targeting of processes involved in TMAO generation may serve as a novel therapeutic approach for atherosclerosis.

oral 025	15 min	lung and cardiovascular diseases	Nichole Reisdorph

Metabolomics in Translational Chronic Obstructive Pulmonary Disease (COPD) Research

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COPD comprises a spectrum of chronic lung diseases and is the third leading cause of death in the United States. Although smoking is the predominant risk factor for COPD, most smokers do not develop COPD. Individuals with COPD present with variable clinical phenotypes including frequent exacerbation, emphysema, and chronic bronchitis. Little is known about what predisposes individuals to develop a particular phenotype. We initially utilized a systems approach to determine that ceramide metabolism is disrupted in severe versus non-severe COPD subjects. Subsequent studies suggest that sphingomyelins are strongly associated with emphysema and glycosphingolipids are associated with exacerbations of COPD. Studies based on a larger human COPD cohort and mouse smoking models demonstrate overlap in several significantly affected pathways. Plasma was collected from current and former smokers (n=129) aged 45–80 years that are part of the COPDGene cohort. Plasma was collected from C57BL/6 mice (n=126) exposed to air or cigarette smoke for 9 months. Metabolomics: Liquid-liquid extraction was performed followed by LC/MS analysis of resulting aqueous and lipid fractions. Mass Profiler Professional and R

were utilized to identify differences in metabolites among groups. Metabolites were annotated using public and in-house databases; identities were confirmed with standards and/or MSn where possible. Sphingolipids: Over 100 species were measured in plasma by tandem MS (n=129). Regression analysis with adjustment for clinical covariates and correction for false discovery rate (FDR) and meta-analysis were used to test associations between COPD subphenotypes and sphingolipids. Current and former smokers were classified based on several variables: smoking status, percent emphysema, airflow, chronic bronchitis, exacerbations, and GOLD status. Mice were placed in the following groups: 0 month control, 3 month +/- smoking, 6 month +/- smoking, 9 month +/- smoking, and 6 month smoking followed by 3 months air control. In a related experiment mice were treated with sphingosine-1-phosphate analogs at 0 months, followed by the treatments listed above. Differentially regulated metabolites were identified using linear regression modeling for the above variables; human data was adjusted for covariates including age, gender, and BMI. Metabolomics: Over 4500 metabolites were detected in both the mouse and human plasma samples. For human datasets, 455 statistically significant metabolites ($p \leq 0.05$) were identified and mapped to fourteen pathways. These include ceramide metabolism and glycerophospholipid metabolism. Comparison of the nine month air control to the nine month smoking mice groups (n=10 mice/group) resulted in 147 statistically significant metabolites ($p \leq 0.05$) in the mouse plasma. Levels of one metabolite, lactosylceramide, return to normal following sphingosine-1-phosphate analog treatment; treatment also resulted in a corresponding attenuation of lung injury. From these combined results, six pathways were common to and significant in both the mouse and human data. Sphingolipids: MS analysis of 69 sphingolipids resulted in strong signal and were used for comparison. These were further reduced to 10 distinct classes based on strong correlations between classes of sphingolipids. The most statistically significant negative (inverse) association between a COPD phenotype and a sphingolipid class was for emphysema and sphingomyelins; the most statistically significant positive (direct) association was COPD exacerbations and trihexosylceramides. Of all detected plasma sphingolipids, 6 sphingomyelins were associated with emphysema, 4 trihexosylceramides and 3 dihexosylceramides were associated with COPD exacerbations. Metabolomics is used to determine subphenotypes of COPD and revealed overlap between human COPD patients and mouse smoking models.

oral 026	15 min	lung and cardiovascular disease	Philipp Schatz

Development of a Metabolomics-Based In Vitro Diagnostic for Early Stage Heart Failure and its Translation into a Clinical Test

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Blood-based biomarkers for heart failure (HF), in particular brain natriuretic peptides (BNPs), are limited by their lack of sensitivity for early HF detection. A marker with improved sensitivity in early HF is needed for screening at the primary care physician and initiating early treatment in particular of heart failure with reduced ejection fraction (HFrEF). We identified metabolites improving established diagnosis, validated the marker and developed an in vitro diagnostic that significantly outperforms specificity of comparable tests. Prospectively recruited subjects consisting of patients with systolic HF (n = 371) and healthy controls (n = 244) were randomly divided into a training set (n=400) and a testing set (n=215). Metabolite profiling of plasma samples was performed by untargeted GC-MS and LC-MS and using a simplified targeted analytical protocol. For the novel HFrEF metabolite-based biomarkers an multimarker assay for application at the primary physician and in high throughput clinical testing was developed. The metabolomic biomarker panel comprises complex lipids (cardiac lipid panel) and NT-ProBNP. It distinguishes patients with systolic HF from healthy controls with an area under the curve (AUC) of 0.97 in the testing dataset (sensitivity 80.2%, specificity 97.6%) and was significantly superior compared to NT-proBNP alone (AUC = 0.93; sensitivity 81.7%;

specificity 88.1%, p < 0.001). The increase in AUC over NT-proBNP alone was particularly pronounced in the HF subgroup with only mildly reduced left ventricular ejection fraction (35% ≤ EF ≤ 50%; 0.94 vs. 0.87, p < 0.001). In further mechanistic studies most metabolic changes could be confirmed in a transverse aortic constriction (TAC) mouse model results of which we will discuss in more detail at the conference. The cardiac lipid panel added significant diagnostic specificity for several HFrEF subgroups on top of NT-proBNP, thereby overcoming the limitations of single feature markers in this complex disease. MS based multimarker finding can be translated into enzymatic high throughput assayable diagnostic applications readily applicable in clinical routine. (1) New metabolomic biomarker for heart failure (2) Technology transfer from discovery to routine use

oral 027	15 min	lung and cardiovascular disease	Yasmeen Nkrumah-Elie * Early Scientist

Sphingolipid metabolism is altered in identical twin pairs that are discordant for asthma

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Asthma is a chronic lung disease characterized by inflammation and narrowing of the airways, resulting in recurring wheezing, tightness of the chest, shortness of breath, and coughing. According to the CDC, nearly one in ten children in the United States has been diagnosed with asthma; and rates continue to increase, particularly in urban communities. There is still little known about the causes of asthma and susceptibility to asthma attacks, though genetic and environmental factors have been considered. Metabolomic analysis of identical twins discordant for asthma may help to elucidate the mechanisms and altered susceptibility associated with non-genetic risk factors for asthma. Plasma samples from ten sets of adolescent, (8-16 years old) monozygotic twins discordant for asthma were prepared for metabolomics analysis by methanol precipitation and liquid-liquid extraction using water and methyl-tert butyl ether. The aqueous and lipid fractions were analyzed by untargeted LC/MS. Chromatographic data was extracted and analyzed using commercial software (Agilent Technologies). An in-house database containing publicly available and in-house spectral data was used to annotate metabolites; annotation was based on accurate mass, isotope ratios, and isotopic distribution with a mass error3000 counts. Unpaired t-tests to compare the metabolites of asthmatic versus non asthmatic twins was conducted, and statistical significance was accepted at p≤0.05 with a fold change of >2. Analysis of LC/MS results in the aqueous (positive ion mode only) and lipid fractions (positive and negative ion modes) yielded 114 significantly different compounds, with 75 compounds increased in the twins with the asthmatic phenotype. A total of 28 compounds had at least a 2-fold change (FC). Preliminary metabolite identification resulted in 30 named metabolites and 24 additional chemical formulas, based upon a minimum database score or overall score of 70, respectively. 4-hydroxy-L-proline, an inhibitor of collagen synthesis and contributor to airway remodeling, a key feature in chronic asthma, was significantly increased (60.73 FC, p=0.022) in the asthmatic twins. 1-phosphatidyl-D-myo-inositol, the primary source of arachidonic acid for the biosynthesis of eicosanoids, the fatty acids involved in the regulation of airway inflammation and reactivity, was decreased by an average of 17% (p=0.029) in the asthmatic twins. Preliminary pathway analysis using MetPA and Mbrole, based upon named metabolites in the data set, listed 8 potential impacted pathways. Sphingolipid metabolism was the most impacted pathway, with a raw p-value of 7.986E-6 and impact score of 0.1545 (MetPA). Three sphingolipids, galactosylceramide (FC=1.25, p=0.035), lactosylceramide (FC=1.17, p= 0.003), and galabiosylceramide (FC=1.15, p=0.012) were all increased in association with asthma, whereas, 2R-amino-3E-octadecene-1,5-diol, also known as 5-hydroxy,3E-sphingosine, was shown to be decreased in the asthmatic twin (FC=1.64, p=0.031). The impact on sphingolipid metabolism confirms previous mouse model research conducted by our group. Asthma concordance in identical twins is 50% and therefore suggests significant environmental rather than strictly genetic causation. Metabolomic analysis of the plasma of identical twins discordant for asthma demonstrates significant small molecule differences between the asthma phenotypes, and can lead to the elucidation of mechanisms associated with asthma pathobiology. This research is novel in the use of metabolomics to elucidate non-genetic factors contributing to asthma susceptibility and etiology.

oral 028	15 min	lung and cardiovascular disease	Dean Jones

High-resolution metabolomics of cardiovascular disease: Linking health exposures and disease phenotype

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Cardiovascular disease (CVD) is a complex process involving genomic, epigenomic and life history variations in diet, environment, microbiome and health behaviors. High-resolution metabolomics (HRM) provides a dynamic integration of these factors. Progress in understanding CVD and underlying mechanisms has resulted in considerable improvements in disease prevention and management, yet early preclinical evaluation and prediction of outcomes remain challenging. This project applies HRM to cohorts extensively phenotyped for subclinical vascular disease (endothelial dysfunction, carotid intima-media thickness, arterial stiffness, and microvascular dysfunction), clinically diagnosed CVD measured as presence/absence of coronary artery disease at angiography and its severity and previous history of myocardial infarction, and a mouse model of CVD induced by disturbed blood flow (d-flow) in the carotid arteries. Acetonitrile-extracts of plasma were analyzed in triplicate using C18 chromatography and high-resolution mass spectrometry (85–1200 m/z). Accurate mass data were extracted with xMSanalyzer using apLCMS. Bioinformatics and biostatistics used online and in-house tools, e.g., MetaboAnalyst 2.0 (http://www.metaboanalyst.ca/MetaboAnalyst/faces/Home.jsp), Kyoto Encyclopedia of Genes and Genomes (KEGG) (http://www.genome.jp/kegg/), the Human Metabolome Database (HMDB) (http://www.hmdb.ca/), Lipid Maps Lipidomics Gateway (http://www.lipidmaps.org/), Metlin and Mummichog. Targeted metabolome-wide association study (MWAS) of significant metabolites and 2-way hierarchical clustering analysis (HCA) were used to complement pathway enrichment methods. Metabolites were identified with MS/MS, coelution with authentic standards, and cross-platform validation. Analyses of biological functions and pathways associated with significant metabolites altered by partial carotid ligation were analyzed by MetaCore (https://portal.genego.com/). MWAS of risk factors for CVD showed subsets of metabolites significantly associated with age, BMI, race, sex, arterial stiffness, oxidative stress markers, pro-inflammatory cytokines, smoking and other risk factors. The richness and complexity of the data, including discovery of correlations with un-identified chemicals, confirms the expectation that metabolomics of CVD will reveal new mechanistic links as well as improved understanding of the metabolic basis of risk-factor associations with CVD. Significant correlations include essential and non-essential amino acids and related metabolites, choline and related metabolites, a range of fatty acid metabolites and other lipids, energy intermediates, vitamin and coenzymes, microbial metabolites and environmental and food-derived chemicals. The range of correlations supports the interpretation that common mechanisms contribute to CVD in a large portion of the population and that uncommon mechanism contribute to CVD in smaller subsets of the population. MWAS of the mouse model of CVD showed that 128 metabolites were significantly altered in the ligated mice compared with the sham group. Of these, sphingomyelin (SM; m/z 703.5747), a common mammalian cell membrane sphingolipid, was most significantly increased in the ligated mice. Of the 128 discriminatory metabolites, 18 and 41 were positively and negatively correlated with SM, respectively. The amino acids methionine and phenylalanine were increased by d-flow, while phosphatidylcholine and phosphatidylethanolamine were decreased by d-flow, and these metabolites were correlated with SM. Other significantly affected metabolites included dietary and environmental agents. Pathway analysis showed that the metabolic changes of d-flow impacted broad functional networks. These results suggest that signaling from d-flow occurring in focal regions induces systemic metabolic changes associated with atherosclerosis. MWAS of Phe further showed complex correlations with estrogens and a range of lipids. High-resolution metabolomics provides a practical approach to obtain detailed metabolic phenotyping for prediction, classification and management of CVD.

oral 029	30 min	Novel use of isotope labels	Darren Creek

Determination of active metabolic pathways in haematopoietic stem cell-derived reticulocytes with stable isotope labelled metabolomics

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Haematopoietic stem cell-derived reticulocytes provide an in vitro model system to investigate red blood cell (RBC) development with relevance to transfusion medicine, RBC diseases and infectious diseases. Of particular interest, most malaria parasites preferentially invade the immature reticulocytes rather than mature RBCs, and it is hypothesised that reticulocytes provide a favourable nutrient-rich environment for parasite growth. In this study, reticulocytes and mature RBCs were compared with untargeted metabolomics, and stable isotope labelling was incorporated to determine the active metabolic pathways in cultured reticulocytes and mature RBCs. CD34+ haematopoietic progenitor cells were purified from blood from human volunteers and cultured in a three-stage protocol to produce cultured reticulocytes at day 18. Isogenic mature RBCs were obtained from matching donors and incubated in identical culture media for 24 hours prior to analysis. For labelling experiments U-13C-glucose (50%) was added to culture media 1 hour or 24 hours prior to extraction. Metabolites were extracted with chloroform/methanol/water (1:3:1 v/v) and samples analysed using LC-MS with HILIC (ZIC-pHILIC) chromatography and high resolution accurate mass (Orbitrap) mass spectrometry. Data was analysed with the IDEOM workflow (incorporating XCMS and mzMatch.R) to determine differentially abundant metabolites from unlabelled samples and to compare 13C-label incorporation into all putatively identified metabolites. Untargeted metabolomics detected over 1100 putatively identified metabolites, including 507 (45%) that were present at significantly different concentrations in reticulocytes compared to mature RBCs. Of these, 136 putative metabolites were greater than 5-fold more abundant in reticulocytes, including metabolites from amino acid, carbohydrate, nucleotide, lipid and peptide metabolism, whereas only 31 features were 5-fold higher in mature RBCs. It was therefore proposed that reticulocytes have significantly greater metabolic activity than erythrocytes, which was tested by coupling stable isotope tracing with metabolomics. Metabolome-wide isotopologue analysis of reticulocytes and mature RBCs after incubation with U-13C-glucose revealed active glycolysis and pentose phosphate pathways in both stages. No significant labelling was observed in metabolites of the related TCA cycle, amino acid and nucleotide pathways in mature RBCs after 24 hours, and only minimal labelling of these metabolites in reticulocytes (<10%) suggests that the major differences observed in the untargeted metabolomics are not due to continual production of these metabolites in the reticulocyte stage. The greatest differences in stable isotope incorporation between reticulocytes and mature RBCs were observed in intermediates of glycan metabolism, revealing greater activity in glycoconjugate biosynthesis pathways in reticulocytes compared to mature RBCs. Stable isotope labelled metabolomics provided mechanistic biochemical insight into the differences observed in the reticulocyte and mature RBC metabolomes.

oral 030	15 min	Novel use of isotope labels	Bernhard Kluger * Early Career Scientist

A stable isotopic labeling assisted approach for the preparation and LC-HRMS based characterization of plants and filamentous fungi

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Modern LC-HRMS based untargeted metabolomics approaches aim at the probing of the global metabolic space of a

biological system and are perfectly suited to study primary and secondary metabolism. The use of 13C carbon and 15N nitrogen offer new possibilities to overcome limitations in this field such as correct recognition of biologically derived features or identification of unknown compounds. However, the major bottleneck of stable isotope assisted approaches is the preparation of labeled plants and fungi, which is needed for global untargeted metabolite profiling. The cultivation of plants in a controlled 13CO2 atmosphere and fungi using U-13C and U-15N labeled media is presented and the benefits and limitations in characterizing the resulting labeled metabolites are discussed. Wheat plants (control and pathogen treatment) were grown from seeds to harvest in a customized labeling chamber either in a controlled native or 13CO2 atmosphere (degree of enrichment >99%). Fungal cultures were grown in parallel in minimal media containing glucose and sodium nitrate either in its native or U-13C (glucose as sole carbon source) or U-15N (sodium nitrate as sole nitrogen source) labeled form, both with a high degree of enrichment (>99%). During sample preparation, aliquots of native and labeled sample extracts were pooled together in the ratio 1:1 (v/v). Subsequently, all samples were analyzed using C18 reversed phase HPLC coupled to an LTQ Orbitrap XL system operated in full scan mode (m/z 100-1000). Resulting U-13C and U-15N labeled metabolites have nearly identical physical and chemical properties as their native analogues. Hence, chromatographic peaks of native and U-13C labeled metabolites show perfect co-elution and have been evaluated automatically with our in house developed MetExtract II software. Under the tested conditions, a LC-HRMS chromatogram contained approximately 2,000,000 MS signals, which can be summarized to roughly 4000-8000 chromatographic features, only 10% of which originate from real biological metabolites. Thus, globally labeled organisms allow for the first time the comprehensive but selective and automated consideration of all features in a biological sample which make up the metabolic inventory that can be detected by LC-HRMS under the tested conditions. Analysis of labeled wheat ears revealed ~900 metabolic features corresponding to ~362 metabolites with an isotopic degree of enrichment of >98% for U-13C labeled metabolites. Different metabolites were observed between control and pathogen treated wheat plants and reveal the importance of equally treated native and labeled plants for the discovery of pathogen induced metabolites. In fungal cultures ~400 metabolic features corresponding to ~90 metabolites were found with a degree of 13C enrichment of >99%. For each detected metabolite ion the exact number of carbon and nitrogen atoms was determined based on both labeling approaches, thereby reducing the number of possible sum formulas, which gives an advantage for the annotation and identification of unknown metabolites. Moreover, with the presented approach, global internal standardization is feasible which allows more accurate metabolome wide comparative quantification. The introduced SIL assisted workflow enables cultivation, sample preparation, LC-HRMS measurement and fully automated data evaluation. The various benefits of the labeling approach can even be further enhanced when the native as well as the labeled organisms of interest are cultured in parallel under both, control and treatment conditions. A stable isotope assisted workflow for the untargeted global detection of customized plants and fungi is presented and critically evaluated.

oral 031	15 min	Novel use of isotope labels	Malcolm McConville

Dissection of the carbon and energy metabolism of parasitic protozoa using 13C-and 2H-stable isotope resolved metabolomics

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The parasitic protozoa are the cause of a number of devastating diseases, including malaria, toxoplasmosis, African sleeping sickness and leishmaniasis, that collectively affect more than half of the world's population. There are no vaccines for any of these diseases, and current drug therapies are limited and/or problematic, reflecting in part, the high degree of similarity between these eukaryotic pathogens and their human/animal hosts. Current efforts to develop new anti-parasite drugs have been hampered by our lack of information of the core metabolism of these pathogens, many of which have an obligate intracellular life-style. New methods are therefore needed to delineate the carbon metabolism of these parasites in situ during both acute and chronic phases of infection. We have developed comprehensive 13C-stable isotope labeling approaches for delineating complex metabolic networks in intracellular stages of several medically important parasites, including Leishmania spp, Plasmodium falciparum and Toxoplasma

gondii. These approaches include (1) rapidly isolating parasites from their host cell and labeling with a suite of 13C-labeled precursors (sugars, amino acids, fatty acids) or (2) labeling parasites inside infected host cells and then isolating the parasites for metabolite analysis. In a recent extension of this approach we have developed methods for labeling intracellular parasite stages with 2H2O. 2H2O labeling provides a universal method for labeling many metabolic intermediates and can be used to measure key physiological parameters (such as replication, transcription and protein translation) of microbial pathogens in infected tissues. These approaches have resulted in substantial revisions of our current understanding of the metabolism of all of these parasites. For example, our studies have highlighted unanticipated complexity in the carbon metabolism of the apicomplexan parasites, T. gondii and P. falciparum. Both parasites were previously thought to utilize glycolysis as their sole source of ATP generation and to lack a functional TCA cycle. However we showed that these parasites are critically dependent on mitochondrial metabolism of glucose for development in the mammalian host and/or transmission. Unexpectedly we find that these parasites are also dependent on several metabolic futile cycles, including cycling between fructose-6-phosphate kinase and fructose-1,6-bisphosphatase to maintain key fluxes into the pentose phosphate pathway and glycoconjugate biosynthesis. We have confirmed that key enzymes in these futile cycles, such as FBP are essential for parasite virulence and suggest that these cycles may have replaced other regulatory mechanisms common in higher eukaryotes, such as transcriptional regulation and post-translational modification of enzymes. We have used the 2H2O labeling to further dissect parasite metabolism and physiology in vivo. Mice infected with Leishmania parasites were provided with 2H2O in their drinking water and incorporation of deuterium into parasite macromolecules (DNA, RNA, protein, lipids) determined at different time points. These studies showed that pathogenic stages of Leishmania enter a slow dividing, metabolically quiescent state in lesions, contrary to expectation, which may account for the intrinsic resistance of these parasites to many drugs. These studies define the metabolism of important human pathogens, and provide novel approaches for studying other host-pathogen systems

oral 032	15 min	Novel use of isotope labels	Christin Zasada * PhD student

Quantifying metabolic fluxes in cancer and stem cells

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Despite their different origin, cancer and stem cells share the feature of fast growth and unlimited proliferation. It is under debate if their mode of proliferation should require likewise distinct demands of precursors for biosynthesis or energy; regardless both cell types favoring aerobic glycolysis. In our lab we combine MS-based ‘omics’-technologies (LC-MS / GC-MS) to deliver relative and absolute quantitative information about enzyme abundance and metabolite concentrations of the central carbon metabolism (CCM). We have developed pulsed stable isotope resolved metabolomics (pSIRM) to monitor how cells utilize substrates like glucose and glutamine to meet their energetic requirements. Finally, the integration of quantitative and time-resolved isotope incorporation in a mathematical framework enables the calculation of the metabolic fluxes; the only functional readout of a cell. Isotopically non-stationary (INST) metabolic flux analysis uses the collected data (absolute pool sizes, extracellular rates) in combination with a network model (material balances, carbon transitions) to jointly estimate intracellular metabolic fluxes and pool sizes in cell culture experiments. The complete workflow – from the petri dish to the metabolic flux map – has been applied to track the rerouting of carbon usage during pluripotency, reprogramming and differentiation. We applied stable isotope labeled substrates and analyzed their fate in fibroblasts and their pluripotent counterparts (iPSC), in breast cancer MDA-MB231 and in human embryonic stem cells (hESC). The analysis endorsed the switch from a glycolytic to respiratory metabolism after differentiation both in hESC- and iPSC-derived fibroblasts. Specifically the comparison of the metabolic profiles of stem cells and cancer cells revealed distinct metabolic features of these cell types. Instationary metabolic flux analysis in cancer and stem cells.

oral 033	15 min	Novel use of isotope labels	Katharina Nöh

Implementing 13C-Metabolic Flux Analysis in an Industrial Environment: Detailed Intracellular Insights in Penicillin V production with P. chrysogenum

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Since the discovery of the antibiotic penicillin in the 1930s, the fungus Penicillium chrysogenum was continuously engineered towards high penicillin yields. Conventional strain improvement, however, has become more and more laborious, resulting in prolonged improvement cycles. The contested global pharmaceutical market situation accelerated the need for a rationally driven strain development that relies on a detailed, integrated understanding of the biological system within its large-scale bioprocess. In collaboration with the Sandoz Company, the industrial penicillin V production process with a high-yielding P. chrysogenum strain was investigated with isotope labeling experiments to derive new knowledge-based hypotheses for strain improvement. Intracellular metabolic reaction rates (fluxes) are key descriptors of cellular physiology and, thus, their quantification is essential to assess the effect of genetic interventions. Recently, metabolic flux analysis using isotope tracers (13C-MFA) has become the method of choice to determine these fluxes in vivo. The classical 13C-MFA workflow comprises several steps: Informative 13C-labeling experiments (ILEs) are designed and conducted under metabolic steady state conditions. Emerging 13C-labeling patterns of intracellular central carbon metabolites and amino acids are extracted and analyzed using state-of-the-art metabolomics devices. The labeling incorporation is described by mass balances and the metabolic fluxes are determined by minimizing the discrepancy between observed and simulated labeling patterns. Finally, a statistical analysis uncovers the significance of the calculated flux map. Classical 13C-MFA, however, relies on several idealizing assumptions that can hardly be fulfilled when applying this technology to a nonmodel organism like P. chrysogenum in industrial environments. By going step-by-step through the 13C-MFA workflow, we exemplify several challenges that have to be tackled on the way from planning ILEs to the interpretation of the final flux map. Our methodological advances include: (1) Informative, yet cost-efficient ILEs were designed for fed-batch cultivation conditions. A comprehensive dynamic bioprocess model accounting for the penicillin biosynthesis was built and used for consistent extracellular rate estimation and for backing the metabolic state assumption. To assess the reliability of the model under process variations, sampling-based global sensitivity analysis techniques were utilized. (2) Free intracellular metabolites were extracted from complex biological samples and the data was adequately pre-processed to provide unbiased labeling patterns, a prerequisite of subsequent model-based analysis. (3) A large-scale, yet sensitive, metabolic network of P. chrysogenum, was built taking intracellular compartmentalization into account. For each reaction carbon atom mappings were implemented. Because the underlying network construction procedures were highly interactive, a visual toolset comprising more than 20 plugins for the visualization software Omix was developed to support the modeler. (4) For flux estimation and statistical assessment of the flux map, the high-performance simulation tool 13CFLUX2 was used. These steps are put into practice for a high-yielding P. chrysogenum strain resulting in the first detailed quantitative flux map for this strain under close-industry conditions. One preliminary finding is the strong influence of the biomass formation on the carbon flux towards the oxidative pentose phosphate pathway, indicating the “yin and yang” of production process and strain optimization. Spurred by the gap between actual product yields and their theoretical maximum, we are currently developing a new computational approach to predict gene knock-outs with improved penicillin V yields. A universal recipe, first of its kind, for establishing state-of-the-art 13C-MFA techniques for nonmodel production hosts in industrial environments

oral 034	30 min	data processing	Timothy Ebbels

A Workflow For Processing and Integrating Multi-Cohort, Multi-Platform, Untargeted Metabolomics Data In Large Scale Metabolic Epidemiology: the COMBI-BIO Project

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As the field of metabolomics advances, large scale studies involving thousands of samples are becoming increasingly common. These efforts present multiple challenges in data analysis, particularly when an untargeted platform is used. Problems such as peak alignment and drift correction are particularly difficult in studies with multiple cohorts and analysis platforms. Therefore there is a need for robust processing pipelines which can ensure reliable, quality controlled data for statistical analysis. The COMBI-BIO project is aimed at detection of metabolic markers of pre-clinical atherosclerosis, and incorporates plasma from 8000 individuals, in 3 cohorts, profiled by 6 assays in 2 phases using both NMR and UPLC-MS. Here we present the COMBI-BIO analysis pipeline and show how it addresses the challenges outlined above. Standard 1-dimensional 1H-NMR spectra were aligned using the RSPA algorithm and normalized using the PQN method. After removing interfering signals, outliers identified using Hotelling's T2 were removed and a cohort/phase adjustment was applied. UPLC-ToF-MS data were acquired using both reverse phase and HILIC chromatography, in both ionization modes. Data were processed with XCMS and features removed if they did not show a linear trend in dilution, or were not found in repeatedly injected pooled (QC) samples. PQN normalization was applied, followed by LOESS based drift correction, again based on QC samples. Features showing poor stability across QC samples were removed. Finally a variance stabilizing transformation was applied. This resulted in 2 NMR and 4 UPLC-MS data sets for each sample. A number of quality assessment metrics were computed to assess the developed pipeline. Alignment of the NMR data was shown to increase the correlation-based aq(0.02) quality measure from 0.330 to 0.400 for CPMG and 0.544 to 0.592 for NOESY data showing that the improvement was present across both large and small peaks. Alignment success was also observed in heat maps of stacked spectra. End-to-end quality assessment of the pipeline was achieved by examining the distribution of Hotelling's T2 values across both biological and QC samples. For CPMG spectra, the interquartile range decreased from 1.43 in raw QC data to 0.68 in processed spectra, while the corresponding change for NOESY spectra was 0.79 to 0.64, indicating a substantial improvement in precision following processing. PCA indicated that phase and cohort differences were no longer present in the final data sets. For the MS data, a reduction in the median coefficient of variation of intensity of 46.3% to 13.3% was observed between raw and processed data in independent (not used for correction) QC samples while the biological samples showed a corresponding reduction of 58.3% to 22.4%. Overall performance of the MS pipeline was monitored using PCA plots and showed that the independent QC samples were much more tightly clustered after the processing pipeline than in raw data. Taken together, these results illustrate that the developed pipeline produces robust and reproducible data across thousands of samples, successfully addressing the challenges of this large multi-faceted study. This is the first workflow for multi-cohort, multi-platform large-scale untargeted metabolomics, which has been shown to produce high quality data.

oral 035	15 min	data processing	Paolo Inglese * PhD student

Semi-supervised analysis of 3D mass spectrometry imaging data-sets for tumour heterogeneity exploration

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Intra-tumour phenotypic heterogeneity in human cancer has been associated with tumour progression, treatment resistance and metastasis developments. 3D Mass Spectrometry Imaging (MSI) of human tissues represents a highly promising approach for probing tumour and tumour-microenvironment heterogeneity of molecular phenotypes (hundreds to thousands molecular readouts) through a sample's depth. 3D MSI of a single tissue section generate tens to hundreds of gigabytes of raw data, requiring advanced computational workflows in order to translate raw data into clinically useful information. Here, we present a high-throughput fully automated computational pipeline based on semi-supervised data mining, aimed to discover, quantitative elucidate and localise different molecular phenotypes within tumour and tumour-surrounding tissues from of 3D MSI data. The presented workflow includes: (a) detection of optimal number of principal components for PCA dimensionality reduction via bi-cross validation[1]; (b) detection of optimal number of clusters using a robust method based on reproducibility of clusters, (c) segmentation of tissue into clusters with a hierarchical semi-supervised probabilistic clustering within tumour and tumour-surrounding tissues, (d) visualisation of clusters and spatial consistency evaluation, (e) extraction of (sub-)phenotype specific molecular ion features, (f) quantitative evaluation of diversity of molecular sub-types based on nearest-neighbour estimation of entropy[2]. The pipeline is applied to a 3D Desorption Electrospray Ionization Mass Spectrometry (DESI) dataset from human colorectal adenocarcinoma sample, comprehending tumour tissue and tumour-surrounding tissues, divided into 51 sections. We show that the proposed pipeline was able to extract unique information on the different molecular subtypes within the tumour and its surrounding tissues. The reduction of the dimensionality through the selection of the optimal number of principal components makes the entire analysis computationally tractable, passing from thousands of features to less than 10. The number of clusters is selected with a high reproducibility accuracy (>98%) using three classifiers (linear support vector machine, k-nearest neighbour, linear discriminant analysis), confirming the presence of substructures within each tissue type. The clustering process, based on a mixture model, delineates unique consistent spatial structures within the tumour and its surrounding tissues, which is beyond histological recognition. Furthermore, the entropy estimation is a reliable measure for the internal heterogeneity of molecular phenotypes within tissues, in particular resulting that the tumour tissue is more heterogeneous from the chemical point of view than the surrounding tissues. Finally, by selecting the most correlated molecular ion features with the segmentation maps, we can differentiate the DESI-MSI profiles for each tissue subtype, founding specific patterns in the range typical of lipids, in particular phosphatidyl-inositols, phosphatidyl-serines, and phosphatidyl-ethanolamine plasmalogens, among others. The proposed pipeline represents an important step towards a fully automated histological exploratory method for the identification of the different tumour subtypes that could be addressed with a specific treatment strategy. [1] Owen, A.B. and Perry, P.O. (2009). "Bi-cross-validation of the SVD and the non-negative matrix factorization". Annals of Applied Statistics 3(2) 564–594. [2] Kirill A. Veselkov, Valeriy I. Pahomov, John C. Lindon, Vladimir S. Volynkin, Derek Crockford, George S. Osipenko, David B. Davies, Richard H. Barton, Jung-Wook Bang, Elaine Holmes, and Jeremy K. Nicholson, "A Metabolic Entropy Approach for Measurements of Systemic Metabolic Disruptions in Patho-Physiological States", Journal of Proteome Research 2010 9 (7), 3537-3544, DOI: 10.1021/pr1000576 Quantification of the degree of tissue heterogeneity and automatic identification of subtypes of tissues for tumour and its surrounding tissues.

oral 036	15 min	data processing	Tomáš Pluskal * Early Career Scientist

Mass Spectrometry Development Kit (MSDK): an open-source Java library for mass spectrometry data processing

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Early developments in mass spectrometry (MS)-based metabolomics suffered from a lack of software tools capable of processing high-resolution MS data. Various teams addressed this issue by developing their own, largely incompatible, frameworks, such as XCMS (Smith et al., 2006), MZmine 2 (Pluskal et al., 2010), mzMatch (Scheltema et al., 2011), Maltcms (Hoffmann et al., 2012), and others. Here we introduce MSDK, a collaborative project to provide a common data model and a library of algorithms that can be shared among multiple software tools. The goals of MSDK are to provide a collaborative platform and facilitate efficient development of novel algorithms as well as fair and thorough evaluation of existing ones. MSDK is developed using the Java platform, which provides OS-independent high-level programming environment. Technically, the design of MSDK follows the footsteps of the

popular and mature Chemistry Development Kit (CDK; Steinbeck et al., 2003) and takes further inspiration from the ProteoWizard (Kessner et al., 2008) and OpenMS (Sturm et al., 2008) projects developed in C/C++. MSDK source codes are hosted in a public on-line repository (GitHub) and distributed under a dual open-source license (LGPL+EPL) to maximize the potential for MSDK utilization in both free and proprietary projects. A set of unit tests and sample data are provided with each MSDK algorithm to ensure its correct operation. MSDK currently provides access to MS data through a comprehensive data model based on Java interfaces. The interfaces represent data both in raw form (mass spectra) as well as in the form of processed objects, such as peak lists or identification results. Implementing classes for these interfaces are also provided, with multiple options for data storage backends, since the size of MS data often exceeds the memory capacity of a computer. Algorithms available in MSDK include raw data import and export, feature detection, peak list alignment, and peak identification through on-line databases and other methods. Among the supported file formats are Thermo and Waters native raw formats, mzML, mzXML, mzData, netcdf, and mzTab. Authors of various existing Java-based software tools, both in academia and in industry, have expressed their strong support for the MSDK project, and a number of existing algorithms have already been transferred into MSDK from other projects. We thus expect this library to become a truly powerful platform for metabolomics data processing in near future. The latest version of MSDK as well as its source codes can be downloaded from the following website: https://msdk.github.io MSDK introduces a collaborative platform that was critically missing in the Java-based MS data processing community.

oral 037	15 min	data processing	Andrew Palmer * Early Career Scientist

Translating molecular information from HR imaging MS data: towards spatial annotation of the cellular metabolome

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Cells from a single lineage can exhibit substantial phenotypic changes due to their development and environment which are reflected in an alteration of the cell metabolism. Conventional bulk analysis of cell populations neglects these differences that can result in an inaccurate assignment of both metabolic activity and phenotype. High Resolution imaging Mass Spectrometry can detect and localise hundreds of metabolites within a sample. Although this has strong potential to provide an insight into biological function and phenotypic disease alterations, limitations are imposed by the size of the datasets generated which can exceed 100 GB. Our work reveals the spatial metabolic profiles correlated with phenotypic changes by employing HR imaging MS and Spatial Metabolomics bioinformatics approach. High-throughput molecular annotation was performed on HR Imaging MS data collected using MALDI-FTICR and MALDI-Orbitrap technologies using our recently developed Spatial Metabolomics approach and the Human Metabolome Database (HMDB). Multiple sample types have been analysed including: thin sections of spheroidal colon adenocarcinoma cell cultures; cells imaged directly from culture; and fresh-frozen human brain. Accompanying LC-MS/MS data confirmed the presence of many of the annotated molecules. Then, we performed bioinformatics analysis on revealed metabolite images by performing uni- and multivariate statistics, examining distributions corresonding to molecular classes, and mapping to metabolic pathways databases with the main aim to reveal the link between phenotypic and metabolic changes. The Spatial Metabolomics approach exploits both spectral and spatial filters, in particular a spatial test for presence, to translate complex spectra containing multiple peaks from the same molecule into images of putatively annotated metabolites. It typically annotated 100-1000 metabolites per imaging MS dataset. Once the data was translated and simplified into molecular annotations statistics was calculated using only the biologically relevant information, in contrast to previous approaches which include full sets of peaks from the spectra. Our approach revealed that in the biological systems explored here cell phenotypes were indeed linked to specific metabolites. For example, despite coming from the same cell line, the spheroid cultures revealed an environmentally determined layered structure due to a decreasing gradient of oxygenation and nutrients between the surrounding media and the spheroid core. Univariate analysis revealed sets of metabolites that were localised to particular layers, illustrating the altered metabolism in the nutrient deficient regions. Within the cellular co-

cultures, subpopulations were observed that were not stable when cultured in isolation. The human brain is well differentiated tissue, predominantly into grey and white matter, which have specific biological functions and therefore different metabolic compositions. An individual human brain section was imaged in both positive and negative mode which, due to the translation of the spectra, we could simply combine into one set of molecular annotations for further analysis. Multivariate statistics on just the annotated metabolites was then possible and provided a clear set of differential molecules between the regions, demonstrating how the Spatial Metabolomics approach simplifies the downstream analysis of HR imaging MS data. Annotated molecules can also be linked to known metabolic pathways in order to visualise which pathways may be active in a particular cellular phenotype. HR imaging MS data was translated to annotated metabolite images which revealed markers of cell phenotype and metabolism.

oral 038	15 min	data processing	Nazanin Zounemat Kermani * PhD Student

Spatially resolved metabolomic phenotyping of stromal tissues in breast, colorectal and ovarian cancer by desorption electrospray ionisation mass spectrometry imaging

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Tumour behaviour is highly governed by structures and cells present in tumour stroma. The ability of stromal elements to directly support tumour growth emphasises the need to find specific metabolic profiles of healthy- and tumour-associated stromal tissues. This study devised a comprehensive metabolic phenotyping data analysis method, using imaging mass spectrometric data from breast, colorectal and ovarian cancer tissues obtained by desorption electrospray ionisation (DESI) MS. Discovery of characteristic metabolic profiles for stromal tissue critically depends on a combination of efficient feature selection techniques and robust classification algorithms to recover molecular ion patterns of diagnostic and prognostic importance. Five feature selection methods and ten supervised classification techniques were examined to produce a list of stromal characteristic metabolic patterns. DESI MSI was used to analyse tissue sections of 261 breast, colorectal and ovarian cancer samples. Tissue sections were stained with hematoxylin and eosin, and analysed subsequently by a histopathology professional. The bioinformatics workflow described by Veselkov et al. [1] was applied to co-register MSI and histological data, to obtain mass spectra of known histological identity. Three wrappers and two filter techniques for feature selection and ten supervised classification schemes were compared. A 3-fold cross-validation scheme was used for parameter tuning and feature selection, with leave-patient-out cross-validation for testing performance of histology predictors. Finally, the lists of ions identified to be important by the majority of these techniques were evaluated and used to generate a set of stromal characteristic metabolites. MSI is capable of creating spatially resolved mass spectral data at high spatial resolution that can be co-registered with histological information. DESI MSI is particularly suited to metabolomics studies as it provides good coverage of many of the low molecular weight constituents of tissue samples. 131 breast, 101 colorectal and 29 ovarian cancer samples consisting of adipose, fibrous, glandular, lymphoid, mucosa, muscle, submucosa, tumour, tumour associated muscle and tumour associated stroma were analysed. Here, tumour tissues are interspaced with adipose, muscle and stromal tissues. We refer to these stromal tissues as tumour associated stroma, as one goal of this study is to find their characteristic metabolites and compare them to characteristic metabolites from healthy counterparts of stromal tissue components. The integrated data analysis workflow consisted of spectral pre-processing, co-registering MSI data and histological data, and supervised classification and feature selection phases. Optimized pre-processing steps include filtering of noise/solvent related peaks, baseline correction, variance stabilizing normalization and accurate matching of peaks across multiple tissue slides. Next, using optical images of the stained tissue sections, a fully qualified histopathologist annotated different tissue types within each sample. Then, MSI data for each tissue slide was overlaid with its optical image to obtain mass spectra of clear histological identity. The final step consists of supervised classification and feature selection. Ten supervised classification schemes based on neural networks, support vector machine, random forest, and linear

discriminant analysis were compared. In addition to the fact that all supervised machine learning methods lead to comparatively high classification accuracies in all three data sets, closer inspection of the results revealed that different multivariate techniques generated overlapping features. Ions consistently identified as being characteristic for stromal tissue may represent a more accurate summary of the stromal-specific ions than any of the data analysis techniques used in isolation. State-of-the-art machine learning and feature selection techniques for automated characterization of distinct metabolite patterns relating to different stromal tissue types.

oral 039	30 min	cancer metabolism in human subjects	Caroline Johnson

Correlating Colorectal Cancer with Biofilm Metabolism

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Colon cancer has numerous risk factors but recent studies have highlighted a potential role for bacterial biofilms in cancer development. Biofilms line the mucosal surface and indicate disruption of the normal colon mucous barrier. We recently demonstrated that biofilms are associated with human cancer and are linked to cancer location with virtually all right-sided colon cancers and paired normal tissues possessing biofilms, while left-sided cancers and normal tissues were infrequently biofilm positive. We hypothesized that biofilm structure affects cancer biology by modulating the metabolome, yielding metabolites that enhance cancer growth. Colon cancers and paired normal tissues were collected from 30 colorectal cancer patients at Johns Hopkins Hospital. Biofilm presence was assessed by fluorescent in situ hybridization (FISH) and scanning electron microscopy (SEM). We used four independent metabolomic platforms to examine the metabolome: untargeted and targeted analyses carried out by LC-QTOFMS and LC-QqQMS. Nanostructure-initiator mass spectrometry (NIMS) imaging identified the spatial distribution of metabolites, and global isotope analysis determined the metabolite fate of a key metabolite. Immunohistochemical (IHC) staining and quantification allowed us to assess mucosal enzymes responsible for metabolite upregulation. Additional samples from patients receiving oral antibiotics, and individuals undergoing colonoscopy screening aided assignment of metabolite origin. Untargeted analysis of colon cancers with and without biofilms revealed the upregulation of polyamine metabolites, in particular N1, N12-diacetylspermine (DAS). Selected reaction monitoring confirmed that DAS was significantly upregulated in the tissues, while NIMS imaging correlated normal and cancer cells with polyamine levels, with enhanced detection of the acetylated metabolites at the mucosal edges of the cancer tissues. The presence of acetylated polyamines indicated an increase in cellular proliferation, indeed Ki67 staining showed a significant increase in proliferation in both normal and cancer tissues with biofilms, compared to those without. The regulation of spermidine/spermine N1-acetyltransferase (SSAT) expression was also examined by IHC; host mucosal SSAT expression was not changed tissues with biofilms compared to those without indicating the bacteria were acetylating the polyamines. Furthermore, tissues examined from patients treated with antibiotics 24h before surgery revealed a decrease in DAS to levels seen in tissues without biofilms, providing additional evidence that bacterial biofilms play a role in upregulating this metabolite. Correlation between biofilm formation on cancers, and a metabolite that may affect the growth of both cancer and biofilm.

oral 040	15 min	cancer metabolism in human subjects	William Wikoff

Pre-diagnostic Biomarkers and Clinical Biochemistry of Lung Cancer in Blood and Tumor Tissue

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Metabolomics has the potential to yield blood and tissue based biomarkers relevant to lung cancer screening and early detection. We applied an untargeted metabolomics approach to identify blood biomarkers in a case control study using pre-diagnostic sera from a large patient cohort of current or former heavy smokers, resulting in a single, highly significant potential biomarker. Blinded validation was performed using an independent set of samples and matched controls. A separate metabolomics analysis of early stage tumor and non-tumor biopsy tissue (within-patient) revealed key perturbations in multiple pathways associated with early stage lung adenocarcinoma. For biomarker discovery, pre-diagnostic sera were collected during the Carotene and Retinol Efficacy Trial (CARET) for lung cancer chemoprevention. Two case control studies were performed, with 100 cases and 199 matched controls for training and 108 cases and 216 controls for validation. The protein pro-SFTPB was assayed by ELISA. A metabolite from HILIC LCMS was identified as diacetylspermine using accurate mass, MS/MS, chromatographic retention time, and isotope pattern, all in comparison to the reference compound, which was synthesized according to published methods. GC-TOF was used to analyze tissue samples (tumor and non-tumor pairs) obtained from patients with early stage (Stage IA and IB) lung adenocarcinoma. Statistical methods included t-tests, Kruskal-Wallis, and ROC curve evaluation, and network construction. Blinded analysis of sera from 100 pre-diagnostic cases and 199 matched controls (with cases and controls matched by age, sex, and smoking status at baseline) yielded increased levels of an unknown compound with case:control ratio = 1.87, p-value = 7.86 x 10-8 ; adjusted p-value = 1.70 x 10-4 after FDR correction. This compound was identified as N1,N12-diacetylspermine (DAS), and achieved statistical significance for total AUC of 0.66, (p = 0.01). DAS levels were significantly higher in samples collected 0-6 months prior to diagnosis than samples collected between 6-12 months prior to diagnosis, suggesting increasing serum DAS levels with tumor development and progression. An independent validation set consisted of 108 cases and 216 matched controls. As with the discovery set, DAS levels were significantly elevated among cases versus controls with AUC of 0.650. DAS significantly complemented the performance of the protein biomarker pro-SFTPB in both the discovery and validations sets with a combined AUC in the validation set of 0.808. For adenocarcinoma tumor tissue analysis, 462 metabolites were observed in 39 malignant and non-malignant lung tissue pairs from current or former smokers with early stage (Stage IA-IB) adenocarcinoma. Key cancer-associated metabolic perturbations in adenocarcinoma compared to non-malignant tissue included: 1) decreased glucose levels, consistent with the Warburg effect, 2) changes in cellular redox status highlighted by elevations in cysteine and antioxidants, alpha- and gamma-tocopherol, 3) elevations in nucleotide metabolites 5,6-dihydrouracil and xanthine suggestive of increased dihydropyrimidine dehydrogenase and xanthine oxidoreductase activity, 4) increased 5'-deoxy-5'-methylthioadenosine levels indicative of reduced purine salvage and increased de novo purine synthesis and 5) coordinated elevations in glutamate and UDP-N-acetylglucosamine suggesting increased protein glycosylation. Distinct metabolic perturbations associated with early stage lung adenocarcinoma which may provide candidate molecular targets for personalizing therapeutic interventions and treatment efficacy monitoring. N1,N12-diacetylspermine is a new serum pre-diagnostic biomarker for NSCLC, complementing the protein biomarker pro-SFTPB for lung cancer screening.

oral 041	15 min	cancer metabolism in human subjects	Elodie Jobard * Early Career Scientist

NMR investigation of targeted therapy effects on the host metabolism for HER-2 positive breast cancer

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Many advances in recent years, such as the use of targeted therapies, have enabled to improve the management of cancer patients. Understanding the effect of targeted therapies on the human metabolism is essential to predict the response to treatments and adjust personalized therapy. mTOR is an attractive target for cancer's therapeutic intervention because of its key role in the regulation of protein translation, cell growth and metabolism. We present

here a metabolomic investigation exploring the impact of mTOR inhibition on serum metabolic profiles from patients with non-metastatic breast cancer overexpressing HER-2. We detail the metabolic signatures associated with the response to an HER-2 inhibitor standard treatment (trastuzumab), or a combined HER-2 and mTOR (everolimus) inhibitors therapy. Pre-treatment, on-treatment and post-treatment serum samples were available for 75 patients with HER-2 positive breast cancer from the French multicenter, randomized phase II trial RADHER. Fasting patients were randomized between two treatments: trastuzumab (group A) and a trastuzumab and everolimus combination (experimental group B). For each patient, clinicopathological data were recorded. 252 serum metabolic profiles were obtained using 1H NMR spectroscopy (800MHz). Unsupervised (PCA) and supervised (PLS and O-PLS) statistical multivariate methods were exploited to build models for sample classification and extract group-specific metabolic signatures. ANOVA analysis and multiple testing correction of the p-values were used to derive statistically significant associations of individual metabolites. Analysis and visualization of involved metabolic pathways was achieved using MetPA and iPath tools. Clinical-pathological parameters including age, BMI, menopausal status, collection centre, hormones receptors, an evaluation of the response to therapy and toxicity were investigated in order to exclude biases related to patient selection. No significant differences were observed for all these parameters between the two arms of the study. The longitudinal series of serum samples available for each patient was exploited to investigate the effect of the everolimus and trastuzumab combination B on the patient's metabolism over time and to compare it with the standard reference treatment A. For each arm, metabolic profiles are compared before, over and after treatment. Similarly, the fingerprints are compared between treatments. For only the experimental group, clear and significant O-PLS discriminations are observed between serum metabolic profiles before and after two weeks of treatment (N: 66, R2Y: 0.703, Q2: 0.452, p-value: 1.76 x 10-5), and after four weeks of treatment (N: 65, R2Y: 0.603, Q2: 0.301, p-value: 0.001 x 10-5). The statistical significance of these two models are assessed by high values of goodness-of-fit parameters R2 and Q2 and by CV-ANOVA p-value < 0.05. In addition, the re-sampling 1000 times under the null hypothesis of the two models validates their discrimination robustness. The trastuzumab and everolimus combination causes faster changes in patient metabolism than treatment A. Analysis of metabolic fingerprints highlights the involvement of several metabolic pathways such as the synthesis and degradation of ketone bodies, beta-oxidation of fatty acids, arginine and proline metabolism, as well as aminoacyl-tRNA biosynthesis. Furthermore, comparison of the metabolic profiles for the two arms (two weeks and four weeks after the beginning of the treatment) shows that everolimus, an mTOR inhibitor, is responsible for the faster host metabolism modification observed in the experimental arm. In HER-2 positive breast cancer, metabolomics shows host metabolism modifications in response to mTOR inhibitor association to standard targeted therapy.

oral 042	15 min	cancer metabolism in human subjects	Teresa Fan

Stable Isotope Resolved Metabolomics (SIRM) on Fresh Human Tissue Slices as a Preclinical Drug Testing Platform

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All preclinical drug testing models have advantages and drawbacks. We have been employing SIRM to elucidate metabolic reprogramming of lung cancer cells in monoculture, in mouse xenograft/PDX models, and in NSCLC patients in situ (1) to determine the influence of the tumor microenvironment. We have now extended the range of models to fresh human tissue slices under defined cell culture conditions, akin to those originally described by O. Warburg (2). This ex vivo tissue model has a paired benign versus cancer design to minimize the influence of genetic or physiologic differences among individuals on metabolism and drug response, while retaining the original tissue architecture and heterogeneity (3). Freshly resected tissue slices from individual patients (approx. 1 mm or less thick and 5-40 mg in wet weight) were incubated in standard cell culture conditions, with gentle rocking to facilitate efficient gas, nutrient and waste product exchange. Tissue slices can be maintained metabolically viable for at least 48 h of incubation. The metabolic activity was determined by measuring the uptake and transformation of 13C and/or 15N-enriched common nutrient tracers such as glucose and glutamine, using high resolution mass spectrometry, GC-MS, and NMR after a period of incubation. Both SIRM and histologic responses to anti-cancer selenium agents were

acquired in paired benign and cancerous tissue slices. Analysis of the slices at different time points by NMR, MS and histology shows that healthy lung tissue slices, both benign and cancerous, retained their architecture and a broad spectrum of metabolic activities. Little increase in necrosis was observed under control conditions for > 24 h. Glucose and glutamine metabolism was reprogrammed in the tumor relative to the paired benign tissues. The paired tissue also showed very different responses to selenium agents when incubated at the IC50 dose established for lung cancer cell lines. Large-scale changes in metabolic activities were observed by SIRM analysis, which was accompanied by necrosis in the cancerous slices, but not in the benign slices, after 24 h of incubation. Distinct responses to drug treatments were also evident in tissue slices obtained from different patients. Supported by 1U24DK097215-01A1, NCI P01CA163223-01A1, and NIEHS 1R01ES022191-01 1. Sellers, K., Fox, M.P., Bousamra, M., Slone, S., Higashi, R.M.,Miller, D.M., Wang, Y., Yan, J., Yuneva, M., Deshpande, R., Lane, A.N., Fan, T. W-M. (2015) Pyruvate carboxylase is upregulated in NSCLC. J Clin Invest. 125:687-698 2. Warburg, O. (1923) Versuche an überlebendem Carcinomgewebe (Methoden). Biochem. Zeitschr., 142, 317-333. 3. Xie, H., Hanai, J., Ren, J.-G., Kats, L., Burgess K., Bhargava, P., Signoretti, S., Billiard, J., Duffy, K.J., Grant, A. et al. (2014) Targeting lactate dehydrogenase-A (LDH-A) inhibits tumorigenesis and tumor progression in mouse models of lung cancer and impacts tumor initiating cells. Cell Metabolism 19, 795–809. Individualized response of human tissues to drugs is obtained, transcending current limitations of drug selection for clinical trials or treatments.

oral 043	15 min	cancer metabolism in human subjects	Ismael Dale Cotrim Guerreiro da Silva

Plasma metabolomics signature predicts breast cancer progression and complete pathologic response after taxane-anthracyclin based neoadjuvant regimen

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To identify predictive blood metabolites signatures of complete pathologic response (pCR) defined as no histopathologic evidence of any residual invasive and/or noninvasive residual in breast or nodes (ypT0/ypN0), along with signatures able to predict stable disease and/or progression (SDPR) defined here as any response less than 30% reduction in the initial tumor volume in response to neoadjuvant treatment for stage III breast cancer. In this prospective (FIG 1), NIH registered, neoadjunvant taxane/anthracyclin-based protocol, we applied a targeted, quantitative mass spectrometry approach (Biocrates, Innsbruck, Austria) to measure the absolute micromolar concentrations of metabolites in plasma samples from 59 stage III breast cancer patients prior to chemotherapy and compared the results with the final tumor volume after chemotherapy. For the detection of discriminative metabolites, training and validation sets were assembled. Blinded-created data was then imported to ROC Curve Explorer & Tester (ROCCET, available at http://www.roccet.ca/ROCCET) for the computer-assisted generation of uni and multivariate Receiver Operating Characteristic (ROC) curves. Complete pathologic response (pCR) and stable disease/progression (SDPR), were observed in 11% (7/59) and in 32% (19/59) of patients respectively. After training and validation sets, two predictive metabolites combinations for pCR have emerged with good discriminative characteristics, the first one with sensitivity=100.00%, specificity= 83.8%, PPV= 44.44% and NPV= 100.00% in addition to a second one with sensitivity=100.00%, specificity= 89.66%, PPV= 57.14% and NPV= 100.00%. The third metabolites combination was able to predict SDPR with sensitivity=100.00%, specificity= 93.10%, PPV= 84.62% and NPV= 100.00%. The identified metabolites are mainly related to glutaminolysis, glycolysis, ether lipids, biogenic amines and mitochondrial function (Fig 2, 3, 4 and 5). Finally, taking into consideration the cancer-intrinsic subtypes of breast cancer our findings were able to discriminate HER2 Tumors (LumB-HER2 and HER2) from patients with Triple Negatives and Luminals A/B with Sensitivity= 86.36%, Specificity= 76.92%, Positive predictive value= 61.29%, Negative predictive value = 93.02%, p-value = 0.002 (1000 permutations) (Fig 6 and 7). Quantifiable metabolic changes might help in the prediction of chemotherapy response and in the identification of intrinsic subtypes.

oral 044	30 min	environment * exposure	Pim Leonards

An in vivo metabolomic approach for developmental neurotoxic effects by pesticides, combining cognitive and motor function effects with metabolomic pathways

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Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. Various recent epidemiological studies have indicated that exposure to low doses of environmental biologically active contaminants during human development can have deleterious effects on cognitive development in childhood. The EU-funded project DENAMIC investigates neurotoxic effects (e.g. learning and developmental disorders) of low-concentration mixtures of pesticides and a number of common environmental pollutants in children. An important aspect is the development of biomarkers for (developmental) neurotoxicity in animal models (rat) using (epi-)genomics, proteomics and metabolomics. In the current study, the aim was to investigate underlying molecular mechanisms of observed effects on behaviour and cognitive function of pesticide exposure in rats, using targeted and untargeted metabolomics. Effects of pesticide exposure on cognitive and motor function in rats has been investigated using different behavioural tests. Rats were exposed to endosulfan, cypermethrin, carbaryl, or chlorpyrifos during the gestation and lactation period. Besides, a control group was exposed to the vehicle solution only. Brain tissues (cerebellum, cortex, hippocampus, striatum) were collected and subjected to targeted and untargeted cross platform metabolomic approaches using LC-HRTOF-MS, GC-HRTOF-MS, and shotgun HRTOF-MS (lipids). The biochemical networks were mapped for males, females and for each brain tissue using MetaMapp and visualized with Cytoscape including the fold changes and p-values. The cognitive and behaviour tests showed that pesticide exposure had effects on anxiety, spatial learning, motor coordination, motor activity, active avoidance, and working memory. These effects were pesticide specific and some effects were gender specific. For example males showed increased anxiety and impaired spatial learning after endosulfan exposure which was not found in females. In general, carbaryl induces fewer effects on cognitive function and motor activity than the other pesticides. The metabolomics approach showed brain tissue and gender specific effects on different metabolic and neuronal pathways. The largest fold changes (more than 10 fold), compared to the control group, were found in cerebellum, and in cortex and striatum metabolites were mainly increased in females while in males decreased by endosulfan exposure. Pathway mapping showed that effects were found on various pathways (e.g. glycerophospholipids, GABAgeric, serotoninergic, dopamergic, histidine/histamine, amino acids, and (purine)nucleosides, pyrimidine). Endosulfan and cypermethrin exposure resulted in effects on the cholinergic pathway which was specific for striatum, and the strongest effects were seen in males. Both choline and acetylcholine increased with endosulfan exposure indicating that effects are associated with effects at the synaptic level of neurons. But also choline as part of the glycerophospholipid pathway was affected by an increase of diethanolamine in hippocampus and an increase of phosphocholine in cortex. Some of the down and up-regulated metabolites might explain the observed behaviour and cognitive effects. For example, a decrease in the brain neurotransmitter GABA in males (hippocampus) was found, but not in females, which correlates to the impairment of spatial learning observed in males but not in females. GABA binds to GABA receptors in neuron's which results in the inflow of chloride ions and outflow of potassium ions in the nerve cell. The combination of cognitive and motor function effects of pesticides in rat with underlying molecular mechanisms using metabolomic pathways.

oral 045	15 min	environment * exposure	Serge Rudaz

Evaluation and identification of dioxin exposure biomarkers using UHPLC-QTOF, multivariate analysis and in vitro metabolism

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Metabolomic approaches offer new opportunities for a deeper understanding of the molecular events related to toxic exposure. The metabolomic strategy presented in this work allowed the investigation of metabolic patterns related to dioxin effects in human and the discovery of a predictive subset of steroids. It presents an original methodology of data dimensionality reduction by using extreme phenotype as a guide to filter (biologically-driven data reduction). Metabolomic experiments were carried out with ultra-high pressure liquid chromatography (UHPLC) coupled to high-resolution quadrupole time-of- flight (QTOF) mass spectrometry. To evidence dioxin exposure in urine, a chemistry-driven feature selection was achieved to focus on steroid- related metabolites followed by the information gained from the urine analysis of Victor Yuschchenko, the former president of Ukraine who was treated at the Geneva University Hospital after it's intoxication with dioxin. Hence, the information gained from this extreme poisoning phenotype was examined. Supervised multivariate data analysis allowed evidencing features, mainly related to bile acids and phase 2 steroid based-structures. These results supported the hypothesis of liver damage and oxidative stress for long-term dioxin toxicity. A subset of 24 relevant urinary markers of acute toxicity including glucuro- and sulfoconjugated endogenous steroid metabolites and bile acids, was hence selected to evidence effects of exposure. This subset of putative biomarkers was first evaluated to characterize a Czech cohort. Urine samples were collected from workers submitted to severe dioxin occupational exposure in the late 1960's. A first OPLS-DA model was built to distinguish steroid profiles based on the 24 biomarkers measured in the Czech cohort and a control group matched for age. No false negative were found and a specificity of about 81.8% obtained. Then, another human independent cohort from France, composed by people that lived many years next to a municipal waste incinerator that was operating between 1968 and 2002, was evaluated. A model with two latent variables, one predictive and one orthogonal, was obtained by cross-validation. A clear separation of the French cohort vs control samples was also observed. Good classification performance was reported with values of 88.0%, 95.2% and 78.8% respectively for global accuracy, sensitivity and specificity. These results tends to corroborate the 24 compounds as an exposure set of biomarkers, but a formal identification was necessary for a better understanding of toxic effects of dioxin. The majority of steroids excreted in urine are phase I and II metabolites. A definite identification in metabolomics is generally achieved by comparison with authentic chemical standards. Unfortunately, only a few steroids from the 24 putative biomarkers subset were commercially available. Among them, DHEAS, androsterone glucuronide, androsterone sulfate, pregnanediol glucuronide were successfully identified (retention time, exact mass and fragmentation spectrum matching). To generate reference material, in-vitro phase 2 metabolism reactions were carried out on selected parent compound. Four biosynthetized conjugates were successfully generated and matched to the compounds detected in real samples; among them, three glucuronides were produced in human liver microsomes and one sulfated steroid was produced. Additional syntheses with other isomeric precursors are under investigation to obtain additional identifications of the remaining postulated biomarkers. An original data dimensionality reduction by using an extreme phenotype and evidence a pattern related to dioxin is presented.

oral 046	15 min	environment * exposure	Sara Tufi * Early Career Scientist

Exploring surface water extract toxicity and its link to pesticides mixture toxicity in Lymnaea Stagnalis by targeted and untargeted metabolomics

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Over the past decades, the presence of manufactured chemicals in the environment has raised concerns because of their potential for sublethal effects on organisms. A current challenge in metabolomics is the study of mixture toxicity of environmental samples. The aim of our study is to perform a metabolomics experiment on an environmental extract and link the specific metabolic patterns to the most active chemicals in the extract. In order to study environmental induced sublethal effects, a metabolomics strategy (targeted and untargeted) combined with an in vitro assay was applied to investigate the metabolic changes in the central nervous system (CNS) of the fresh water snail Lymnaea stagnalis. Snails were exposed to the environmental extracts, single pesticides and a pesticide mixture.

Surface water from a Dutch agricultural area was sampled with a large volume solid phase extractor using neutral, anionic and cationic sorbents. The extracts of these cartridges were screened for 194 chemicals and their toxicity was tested with acetylcholine esterase (AchE) inhibition bioassay. L. stagnalis specimen were exposed to 1) the most toxic fraction, 2) the most abundant pesticides found in the extract, and 3) the mixture of these pesticides. To identify active unknown compounds in the neutral cartridge, an on-line HPLC-ToF microfractionation combined with the AchE bioassay was applied. The metabolic alteration of L. stagnalis CNS were assessed by untargeted and targeted metabolomics (neurotransmitters). Biochemical networks were built based on metabolites FDR adjusted p-values and fold changes. The chemical targeted analysis on the neutral, anionic and cationic cartridge extracts revealed that most of the pollutants were retained by the neutral cartridge and most of them are pesticides. The most abundant chemicals in the neutral cartridge are two neonicotinoids pesticides (thiacloprid and imidacloprid) and pirimicarb, a carbamate pesticide. The levels of these chemicals in the original surface water concentration are above the normative levels. The toxicity of the cartridge extracts were screened with the AchE bioassay, especially suited to detect organophosphate and carbamate pesticide induced toxicity. The toxicity screening showed that only the neutral cartridge induced inhibition of the enzyme acetylcholine esterase. The neutral cartridge extract was the most active/toxic and was used to expose L. stagnalis. The untargeted chemical screening of the neutral extract revealed the presence of other neonicotinoid pesticides as acetamiprid and thiamethoxam. The in-vitro AchE guided microfractionation enabled the localization of active/toxic fractions. Carbendazim, a benzimidazole fungicide, has been potentially identified online by HPLC-TOF-MS in one of the most active fractions in which the highest inhibition of AchE was observed. The targeted metabolomics on L. Stagnalis CNS exposed to the neutral extract of the field sample highlighted the involvement of the serotonergic pathway. A significant decrease (three-fold change) was observed in the levels of the serotonin metabolite, 5-hydroxyindoleacetic acid (5-HIAA). The same trend was found for snails exposed to imidacloprid at a similar concentration level as the neutral extract. Untargeted metabolomics and biochemical network mapping enabled the discovery of more pathways related to the neutral extract exposure. For instance, significantly increased levels of creatinine, putrescine and betaine were found. Betaine is linked to the choline pathway which is could be related to the pesticides exposure. The toxicity of an environmental extract was tested and linked to the metabolic changes found with single and mixture compounds.

oral 047	15 min	environment * exposure	Sandra Gouveia-Figueira * Early Career Scientist

Shifts in Human Lung Lavage and Plasma Lipidome in Response to Biodiesel Exhaust Exposure Investigated by Quantitative Tandem Mass Spectrometry

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Air pollutants, such as combustion emissions, influence the environment and cause adverse health effects, such as lung diseases, heart attacks and cancer. These adverse effects, combined with increasing prices of crude oil, have accelerated the search for new fuel alternatives such as biodiesel. Circulating bioactive lipid levels (oxylipins and endocannabinoids as a subset of the lipidome) are affected by air pollution exposure, but little is known about the effects of biodiesel exhaust exposure. Oxylipins are inflammatory mediators, comprising traditional eicosanoids derived via the cyclooxygenase and lipoxygenase pathways, and epoxides and diols produced by cytochrome P450 and soluble epoxide hydrolase. Among endocannabinoids, the cannabinoid receptor ligands anandamide (AEA) and 2-arachidonoyl glycerol (2-AG) are well studied. They participate in many pathophysiological processes. This lipidomic profiling was part of a controlled human exposure chamber study. Healthy subjects (N=15) were exposed on two separate occasions for 1 hour to biodiesel exhaust and filtered air (double blinded). Blood samples were collected before exposure and at 2, 6 and 24 hours post-exposure and lung lavage fluids (bronchial wash (BW) and bronchoalveolar lavage (BAL)) were collected by bronchoscopy at 6 hours post-exposure. Samples were prepared in one single solid-phase extraction step and the isolated endocannabinoids and oxylipins were quantified by previously validated ultra-performance liquid chromatography coupled to electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) methods. Sensitivity was higher with two separate injections (one in positive and the other in negative mode) using dynamic multiple reaction monitoring (MRM) scan method. Targeted metabolomics UPLC-ESI-MS/MS (MRM

mode) was used to profile endocannabinoids (N=15) and oxylipins (N=42) in both plasma and lung lavage fluids. Plasma, BW and BAL have previously been used to identify shifts in bioactive lipid profiles in control vs. disease groups. However, the local and systemic effects on oxylipins and endocannabinoids in response to biodiesel exhaust exposure are unknown. Furthermore, little is known about endocannabinoid levels in lung lavage fluid, especially in BW. In plasma samples, 34 oxylipins were quantified with levels ranging from 2.9 pM – 27 nM. 8-HETE, 13-HODE and 12-HETE were the most abundant compounds. A special remark was the detection, in some samples, of Resolvin D2, an important inflammation-resolving mediator. Oxylipins levels in BAL, of the 28 quantified compounds, were 0.27 – 413pM and in BW 0.39 – 626 pM. The endocannabinoid profiling in plasma showed 13 compounds, including 2 prostamides, in the range of 0.05 – 10.5 nM. Fewer compounds were detected in BW and BAL than in plasma, and a larger variation was observed. The levels found in BW and BAL were 7.4 – 344pM and 0.37 – 181pM, respectively. PEA and SEA were the most abundant among the endocannabinoids. Two-way ANOVA with Tukey's multiple comparison test was used to detect significant differences (at α=0.05) in the temporal trends for each compound analyzed in plasma samples, and t-test was used to compare the differences between different exposures at each time point, as well as to detect any exposure-dependent differences in BAL and BW. For instance, PGE2 levels were shifting to a significant extent at different time points after each exposure in plasma. Furthermore, the PGE2 levels were significantly elevated after biodiesel exhaust exposure, both in BW and BAL. A complete statistical evaluation (uni- and multivariate) and biological interpretation of the results are currently undergoing. First comprehensive quantitative profiling of human plasma and lung lavage lipidome (endocannabinoids and oxylipins) after controlled biodiesel exhaust exposure.

oral 048	15 min	environment * exposure	Douglas Walker * PhD student

High resolution metabolomics to identify PBB 153 exposure associated metabolic alterations in vivo

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While traditional epidemiology studies evaluate the association between possible environmental exposures and a measurable health outcome, it is challenging to elucidate the in vivo metabolic contributions leading to disease pathophysiology. Incorporating high throughput metabolomics within population based studies has the capability to link biological response to exposure and metabolic changes contributing to health outcome. This can be accomplished through application of a metabolome wide association study (MWAS), which aims to identify dose dependent changes in metabolite expression and metabolic pathways. To evaluate the efficacy of this approach, metabolomics and targeted biomonitoring analyses were applied in tandem to evaluate metabolic alterations associated with organohalogen exposure. Plasma was obtained from 128 individuals belonging to a multi-generational cohort exposed to a mixture of polybrominated biphenyls (PBB). The most predominant congener, PBB-153, was quantified using gas chromatography/mass spectrometry (GC-MS) with solid phase extraction and isotope dilution quantification. Metabolic profiles were detected using high resolution MS and C18 chromatography following sample preparation by protein precipitation. Body burden of PBB-153 was mapped to metabolic alterations by correlating each m/z feature pairwise to PBB-153 plasma levels. Associations were considered significant if Spearman $|r| \geq 0.3$ and FDR p values ≤ 0.05. The influence of confounding variables was also explored by accounting for age at exposure, age, body mass index and sex. PBB-153 whole plasma concentrations ranged from <0.007 (limit of detection; LOD) to 164.5 ng/mL, with a mean concentration of 2.63 ng/mL. Individuals with plasma levels above LOD were used to evaluate dose depended changes using the 9,031 unique features detected within the metabolic profile. Both positive (increased expression) and negative (decreased expression) associations were observed with PBB-153 levels, which identified 90 features meeting the significance threshold. Annotation of the m/z values indicated strong associations with sphingomyelins and carnitines, in addition to other fatty acid metabolites. Accounting for confounding variables provided identification of additional metabolites associated with PBB-153, which included OH-polychlorinated biphenyls and lipid products. Global metabolic alterations were evaluated using the 90 significant metabolites in a second level MWAS with pathway level enrichment. At a significance threshold of $p \leq 0.05$, 15 metabolic pathways were found to be associated with PBB-153. These included pathways reflective of the

m/z features selected with the initial MWAS (fatty acid activation/metabolism, omega-3 fatty acids, linoleate metabolism), in addition to changes in other important liver associated pathways (cholecalciferol metabolism, urea cycle and drug metabolism). Alterations in immune related pathways were also observed, including arginine, histidine and biopterin metabolism. Evaluation of individual metabolite changes with PBB-153 plasma levels indicated positive and negative associations, suggesting that pathway level alterations occur through changes in central enzymatic processes. This is consistent with the observed changes in fatty acid related metabolic pathways. Hepatic cytochrome P450 levels strongly influence fatty acid metabolism, which also contribute to the primary detoxification pathway for PBBs. These results indicate that PBB-153 associated metabolic alterations can be detected within the metabolome for profiling of exposure related bioeffect.

oral 049	30 min	plant DB and plant systems biology	Mark Sumarah

Discovery of novel antibiotics from fungal endophytes by comprehensive LC-MS based metabolomics

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Secondary metabolites produced by fungal endophytes of grasses and conifer trees have been shown to play an important role in defense of the host against insect and fungal diseases. The discovery of new bioactive compounds is of great interest as endophytes have been proven to be an excellent source of diverse and potent biological agents. In this study, we applied a metabolomics based approach to the investigation of secondary metabolites from fungal endophytes of grapevines, raspberry, blueberry and cranberry plants. This work has led to the discovery of novel antibiotics without the use of traditional bioassay directed fractionation. 188 fungal endophytes were isolated from blueberry, grapevine, cranberry and raspberry leaves collected in Eastern and Central Canada. These fungi were fermented in liquid culture for six weeks, extracted with ethyl acetate and screened for antifungal and antibacterial activity. Extracts were analyzed by LC-MS and spectral libraries of deconvoluted MS and MS/MS spectra were compiled. Data were compared to our in-house library for matches and screened against AntiBase 2013, Dictionary of Natural Products and NORINE for rapid dereplication of known compounds. XCMS and CAMERA were used to process all 188 extracts, while PCA and OPLS-DA plots differentiated and grouped the extracts with SIMCA-P+12. Compounds of interest were isolated by LC-SPE and characterized by LC-MS, NMR and X-ray crystallography. Bioactivity testing on the extracts showed that 56 were active against gram-positive bacteria, 9 against gram-negative bacteria and 47 were antifungal. Comprehensive LC-MS metabolomic screening of the 188 fungal endophyte extracts was used to build libraries of deconvoluted LC-MS data. This allowed for the rapid identification of known and unknown compounds in the extracts. PCA and OPLS-DA analysis of the entire dataset provide a number of unique outlier extracts as targets for new compound discovery. The major components from these extracts were isolated by LC-SPE. Characterization of these isolated compounds by NMR and X-ray crystallography has to date led to the discovery of 9 novel, 8 new and 30 previously reported bioactive compounds. This work demonstrated that fungal endophytes from Canadian fruit bearing plants are an excellent source of novel, new and known fungal secondary metabolites with important bioactivity. All of the known compounds produced by these endophytes are biologically active and remarkably, many have been evaluated for their use as pharmaceuticals. The bioactivity of these compounds suggests that they play an important role in defense against diseases and pests and could be used in agriculture for crop protection, while some may have the potential to be medicinal agents. LC-MS chemometric screening of fungal endophyte secondary metabolites led to the discovery of novel antibiotics.

oral 050	15 min	plant DB and plant systems biology	Ilana Rogachev

Advancing Metabolite Identification in Plant Extracts by the Use of a Large-Scale Reference Library

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Unambiguous metabolite identification is an essential but yet unresolved problem in mass spectrometry-based metabolomics assays. It is particularly critical in studies of rich metabolic matrices such as those present in plant extracts. Generating a comprehensive mass spectra library from highly pure reference compounds isolated from an extensive repertoire of plant species is currently the most reliable strategy for advance in MS based identification of natural products. We injected a comprehensive set of more than 3500 plant metabolites (mostly specialized metabolites) using a UPLC-qTOF MS in the MSE mode in order to generate a large and structurally diverse MS spectra library – termed the 'WEIZMASS' library. Two computational pipelines were developed to deal with the task: one allows automated construction of such a library from injections of chemical standards pools, and the other termed 'MetaboMatch', allows for efficient and accurate matching of high resolution experimental LC-MS data to the library spectra. 'MetaboMatch' represents a multi-modular approach: it uses nine modules which take advantage of the various data features obtained from chromatography and high-resolution mass spectrometry. The software pipeline automatically converted and inserted experimental data of over 3300 pure chemical standards into the 'WEIZMASS' digital MS library. The 'MetaboMatch' software pipeline was initially validated by random sampling and comparison to manual annotations, next by the spiking of 200 chemical standards derived from the 'WEIZMASS' library into a complex biological matrix, and finally through a series of non-targeted annotations in three distinct, model and 'exotic' plant species. Application of the 'MetaboMatch' and the 'WEIZMASS' library resulted in the unambiguous identification of several dozens of metabolites in different plant extracts; in many cases novel structures or those found in only in single or few species to date. Presentation Highlights: In this presentation we will demonstrate the experimental methods used for the rapid generation of the 'WEIZMASS' library: the main considerations in sample preparation and choice of instrumentation parameters for the rapid construction of such a large MS reference library. We will highlight the building blocks of the software pipelines used and, without going in depth into the computational part, present the rationale behind the computational algorithms and the considerations that were utilized for calculating the 'MetaboMatch' scoring function. We demonstrate a novel way for rapid generation of LCMS library and a novel multi-modular software pipeline for metabolite annotations.

oral 051	15 min	plant DB and plant systems biology	Feng Qiu * Early Career Scientist

Development of Plant Metabolite Annotation Toolbox (PlantMAT) to Increase the Chemical Space and Identification Confidence for Plant Metabolomics

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Metabolite identification is the major challenge in metabolomics and it is critical for providing a biological context to metabolomics data. LC-MS is growing in popularity for metabolomics studies due to its high sensitivity, high throughput, and good metabolite coverage. While LC-MS can generate very valuable metabolomics information, the analyses of large-scale and complex metabolomics datasets remains challenging. We have developed a sophisticated, user-friendly software called Plant Metabolite Annotation Toolbox (PlantMAT) to facilitate the automated analysis of the UHPLC-MS/MS data. The identification methods incorporate concepts of "top-down" and "bottom-up" omics approaches for tandem mass spectral annotation and metabolite structural prediction. In addition, orthogonal datasets, including LC retention, MS, and MS/MS, are used for an enhanced identification confidence. PlantMAT software was developed with Visual Basic for Applications 7.0 (VBA) in Microsoft Excel 2010. The algorithms for each step of the identification workflow were programmed as independent, interchangeable VBA-coded modules. A user-friendly interface was designed and implemented for easy utilization of the software. Metabolite profiling of 80% aqueous methanol extracts from the model legume plant Medicago truncatula was performed using a Waters ACQUITY UHPLC coupled to a Bruker maXis impact QTofMS/MS system. The acquired data were initially processed using Bruker's Compass DataAnalysis 4.1 software for peak deconvolution and formula prediction. Metabolite structures or substructures were predicted using PlantMAT. The putative identifications were then confirmed via 1D/2D NMR following UHPLC-MS-SPE purification and concentration of the target metabolites. This initial priority for PlantMAT development was to enhance the identification of plant secondary metabolites, mainly

saponins and flavonoid glycosides. Other compound classes are being added on a continual basis for enhanced coverage. A database was initially constructed within PlantMAT and populated with triterpenes and flavonoids that have been previously identified in Medicago species, including their common names, chemical classes, molecular formula, exact masses, and SMILES string identifiers. The “top-down” metabolite identification process begins with the import of a list of empirically measured UHPLC retention times, accurate masses and elemental formulas for each analyte. The imported analyte list is then queried against a database of known and in silico predicted compounds that are generated through combinatorial glycosylation of each of the various aglycone structures contained within the database. The retention time for each candidate is further predicted using an artificial neural network (ANN) model, and the candidates predicted within 0.5 min accuracy are considered putative identifications. Finally, tandem mass spectra for the putative structural matches are predicted based on the neutral losses of sugar substructures. The predicted m/z values are compared against the experimental measured MS/MS spectra to provide additional evidence of structure. As an alternative for the “top-down” workflow, the tandem mass spectra can be initially annotated based on user-defined fragment ions, neutral losses, and/or m/z differences. This “bottom-up” approach enables the prediction of substructures without the need of combinatorial prediction and database searching. The software tools described above were used for large-scale metabolite identifications in the model plant Medicago truncatula, and the structures for approximately 100 saponins and flavonoid glycosides were efficiently predicted. More than 30 of the putative identifications were confirmed using UHPLC-MS-SPE-NMR, validating the accuracy of PlantMAT. The results also demonstrate that automated and orthogonal analyses of LC-MS/MS data allow for higher-throughput and higher-confidence metabolite identifications. A sophisticated, user-friendly software toolbox called PlantMAT employing “top-down” and “bottom-up” methods for systematic annotation of plant metabolomes was developed.

oral 052	15 min	plant DB and plant systems biology	Atsushi Fukushima

Development of metabolite-profiling database in Arabidopsis: AtMetExpress – Meta-analysis of metabolome data

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Although metabolite profiling experiments are becoming routine in many research groups, making the studies remains laborious and expensive. Therefore the number of samples and conditions analyzed in individual projects is still relatively small and mostly ranges from only two to few hundreds of samples. While the metabolomics community is working towards the setup of sharing metabolome data, mining publicly available information and demonstrating the richness of integration of multiple metabolome datasets remain largely unexploited. The aims of this study are (1) to establish a new web-based platform for metabolome meta-analysis and (2) to use these sets of combined metabolite levels to explore the diversity of complex metabolic networks in the model plant, Arabidopsis thaliana (Arabidopsis). All of the metabolome data were from the RIKEN Plant Science Center (or Center for Sustainable Resource Science). A correlation heat map and a hierarchical cluster analysis were generated using statistical R software (http://cran.r-project.org/). A principal component analysis was constructed using the pcaMethods package. Constructing our interactive analysis tools and visualization was developed by the Shiny package in R (http://www.rstudio.com/shiny/). Here we constructed our database, called AtMetExpress (http://prime.psc.riken.jp/AtMetExpress/), to store the information. It is freely available and includes detailed information about small molecule metabolites detected in Arabidopsis and a small and simple GUI tool for performing meta-analyses, allowing easy metabolome meta-analysis for plant biologists. We demonstrate how meta-analysis of metabolite profiling experiments can be used to generate novel insights into metabolic behavior in a single organism, across a wide range of experimental conditions. AtMetExpress gives a way to access Arabidopsis metabolome data and can facilitate the development of new data mining tools.

oral 053	15 min	plant DB and plant systems biology	Katrin Brauner * PhD student

Signal processing as a systems biology tool to uncover diurnal regulation of sink-source interactions based on metabolite profiles

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Signal processing is a well-established scientific method (e.g. in communication engineering), capable of extracting periodic information from noisy signals exchanged between sender and receiver. We used signal processing techniques to uncover the impact of light periodicity in sink-source interactions. In this approach, periodicity of light is the input signal using photosynthesis as transducer for metabolite profiles and phloem transport to produce biomass and respiration of shoot and root as outputs. Based on diurnally resolved metabolite profiles of leaves, phloem content and roots, signal processing reveals not just time-discrete information to be mapped, but yields dynamic profiles of communication between sinks and sources. Full diurnal profiles recorded for shoot assimilates (carbohydrates, carbonic acids and amino acids), phloem transport, root metabolites, net photosynthesis and respiration of shoot and root were interpreted as time-discrete signals. Power spectral density analysis based on an enhanced discrete Fourier transformation method was used to identify periodic elements of full diurnal profiles. Discrete auto- and cross-correlation showed the conformity of curve shapes for time series data of various metabolites taking possible time delays into account. Correlation-coefficients for curve shapes were calculated to yield information on metabolic network dependencies and relationships between metabolic states and flow rates including phase shift information. This strategy was applied to various metabolic mutants of Arabidopsis thaliana. Major metabolic pathways in leaves of Arabidopsis thaliana, i.e. photosynthesis, amylogenesis and carbon deposition in biomass, were found to highly correlate with the on-off-rectangle of light in a growth chamber. However, phloem transport resembled an unsymmetrical sinus, causing a continuous rise of sink-supply during day and a corresponding decline during the night. Restricted metabolic flux through sucrose changed the transport pattern into rectangular with a four hour time-lag referring to the light phase. This newly gained direct correlation of transport with photosynthesis caused re-programming in other pathways, with e.g. carbonic acid patterns now being correlated with starch turnover and assimilation rate. Interestingly, even growth kinetics were modified, causing a substantial time-of-day delay in maximal growth rate. Disruption of starch synthesis in the well-known pgm mutant also resulted in a correlation of carbonic acid patterns with photosynthesis, without a time delay. Because roots serve as sinks for non-utilizable assimilates in the shoot, the phloem transport pattern in pgm corresponds to an equilateral triangle with a peak in the second half of the light period. This causes an immediate coupling of root metabolite levels with metabolite profiles of the shoot, which is not observed in the wild type. Combining metabolic profiling with information technology methods not only allows for illuminating dependencies within metabolic pathways, but also identifies relations between pathways that do not share metabolites. Thus, the technique will foster deciphering of large networks that by now are inaccessible to conventional systems biology approaches because of arbitrariness in complex networks. A new technique of combining information technology methods with metabolic profiling allows for resolving pathway relations in complex metabolic networks.

oral 054	30 min	pharmacometabolomics	Mohamed Shahin * PhD student

Integrating Metabolomics, Transcriptomics, and Genomics Reveals Novel Signatures Associated with Hydrochlorothiazide Blood Pressure Response

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Hypertension is the most common chronic disease worldwide affecting about one billion people globally, and one third of American adults. It is a significant contributor to heart attack, stroke, and kidney failure, making its control of critical importance. Hydrochlorothiazide (HCTZ) is among the most commonly prescribed first line antihypertensives in the US with over 100 million prescriptions annually, yet, less than 50% of HCTZ treated patients achieve blood pressure (BP) control. This poor BP control reveals that the current approach for therapy selection and BP control is suboptimal. Accordingly, the aim of the current study was to identify novel biomarkers associated with HCTZ BP response by integrating the genomics, transcriptomics and metabolomics profiles of patients treated with HCTZ. This study included 228 white hypertensive participants, from the PEAR (Pharmacogenomic Evaluation of Antihypertensive Responses) study, with BP determined at baseline and after 9 weeks of HCTZ treatment. Genome-wide genotyping was determined via Illumina© Omni-1M-Quad Chip. Untargeted metabolomics analysis was performed on baseline fasting plasma samples using a GC TOF-MS platform. Illumina© HiSeq 2000 was used to conduct whole transcriptome sequencing on PEAR white baseline blood samples of extreme HCTZ BP response (25 responders and 25 non-responders). RNA-sequencing reads were aligned to the reference genome (homo sapiens Hg19) with TopHat2, and gene expression levels were calculated using cufflinks/cuffdiff. Ingenuity pathway analysis was conducted to integrate the BP genome-wide signals at P<1x10-4 with the top significant metabolomics/transcriptomics signals (FDR<0.05). Metabolomics analysis revealed 212 metabolites, of which 13 were significantly associated with HCTZ systolic and diastolic BP response (FDR<0.05). Integrating those 13 significant metabolites with the top signals from the genome-wide analysis (43 genes at p-value<1x10-4) identified the actin nucleation pathway as the top significant pathway (p-value=2x10-4), with Rho kinase1 (ROCK1) and the Vasodilator Stimulated Phosphoprotein (VASP) genes overlapping with arachidonic acid in the same pathway. rs8085654 was the top significant genetic variant in ROCK1 where variant allele carriers had a poor HCTZ BP response vs non-carriers (ΔSBP/ΔDBP: -5.8/-2.7 vs -10.7/-5.9 mmHg, respectively, ΔSBP p=5x10-5 and ΔDBP p=9x10-4). Additionally, rs10995 was the top significant variant within the VASP gene where variant allele carriers had a good HCTZ BP response vs non-carriers (ΔSBP/ΔDBP: -12.3/-8.2 vs -6.8/-3.5 mmHg, respectively, ΔSBP p=1x10-4 and ΔDBP p=3x10-5). Moreover, ROCK1 and VASP baseline expression levels were significantly different between HCTZ BP responders vs non-responders (ROCK1 p-value=0.013, VASP p-value=0.01). Transcriptomics analysis also revealed 14 novel genes with significantly different expression levels between HCTZ BP responders vs non-responders (FDR<0.05). Interestingly, integrating those 14 significant genes with the top signals from the genome-wide analysis revealed, again, the actin nucleation pathway as the top significant pathway (p-value=9x10-6), with the ROCK1 and the VASP genes overlapping with Ras homolog family member B (RhoB; RNA-seq p-value=5x10-5) and CDC42 effector protein (CDC42EP2; RNA-seq p-value=5x10-5). In summary, our results suggest that ROCK1 and VASP might be important determinants of HCTZ BP response. Additionally, integrating genomics with metabolomics and transcriptomics of HCTZ treated patients identified the actin nucleation pathway as a novel pathway that might be significantly involved in the mechanism underlying HCTZ BP response. In conclusion, the results of this study highlighted the strength of using multiple omics for the identification of novel biomarkers and pathways significantly associated with HCTZ BP response. Integrating metabolomics, transcriptomics and genomics is a promising approach for the identification of novel biomarkers/pathways underlying drug response.

oral 055	15 min	pharmacometabolomics	Balmiki Ray

Genetic Variation in the Aryl Hydrocarbon Receptor (AHR) Gene and Kynurenine Levels in Major Depressive Disorder: Pharmacometabolomics-Informed Pharmacogenomics

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Major depressive disorder (MDD) is one of the most common and most debilitating psychiatric disorders worldwide and contributes significantly to increased Disability Adjusted Life Years (DALYs). Selective Serotonin (5HT) Re-uptake Inhibitors (SSRIs) are the most common drugs used to treat MDD. However, only a third of patients treated with SSRIs achieve complete remission. Both 5HT and kynurenine (KYN) are metabolites of the amino acid tryptophan (TRP), and the balance between 5HT and KYN is thought to be important in both the pathogenesis and therapeutic response for MDD. We have evaluated the possible contribution of genetic variation to KYN plasma concentrations in MDD by employing a pharmacometabolomics-informed pharmacogenomic (PGx) research strategy We analyzed 37 metabolites, including TRP and KYN, in plasma samples from 306 patients enrolled in the Mayo SSRI PGx study at both baseline and post treatment using an LCECA platform. We then performed genome-wide association studies for these metabolites both at baseline and after SSRI therapy. We then performed functional genomic studies of variants associated with plasma KYN concentrations. Use of a “Pharmacogemetabolomics-informed Pharmacogenomics” research strategy made it possible for us to identify genetic variants across AHR (top SNP rs17137566; p=6.22E-06) that were associated with baseline plasma KYN levels. These SNPs (observed and imputed) were eQTLs for the AHR gene, both in brain and in LCLs. Functional evaluation using human glioma and human iPSC-derived neurons revealed that knocking down AHR increased both mRNA and protein levels of the AHR-repressor (AHRR) as well as proteins encoded by the IDO1 and TDO2 genes, enzymes that catalyze the rate-limiting step in KYN biosynthesis. Levels of secreted KYN in conditioned media for these cells were also increased. AHRR over-expression resulted in significant increases in AHR, IDO1, TDO2 mRNA expression and KYN levels, indicating a possible feedback loop between AHR and AHRR. ChIP assays revealed increased binding of AHRR to XREs in TDO2 and IDO1 genes after knock down of AHR, indicating that AHRR might act as a transcription factor for IDO1 and TDO2. These findings indicate that genetic variance in AHR may be associated with MDD pathophysiology or response to therapy.

oral 056	15 min	pharmacometabolomics	Laura M. Yerges

Genome Wide Association Analysis with Amine Metabolites Reveals Novel Loci Impacting Human Metabolomic Profiles

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Serum metabolite levels provide critical information for understanding the complex mechanisms which undergird physiological processes and drug response. The field of pharmacometabolomics has shown great promise to identify biomarkers that can predict disease progression and better inform treatment outcomes. However, little is known about the genetic determinants and heritability of metabolite levels. Increased understanding of genetic and environmental contributions to metabolite levels may help better characterize disease etiology and response variability. We measured 42 amine metabolites in 165 Old Order Amish participants from the Pharmacogenomics of Anti-Platelet Intervention (PAPI) Study using liquid chromatography electrochemistry array (LCECA). PAPI participants underwent an 8 day clopidogrel intervention (300 mg loading dose followed by 75 mg daily for 7 days), with baseline and post-clopidogrel blood draws and platelet function testing. Subjects were genotyped using the Affymetrix 500K or 6.0 chip and the Illumina Human Exome BeadChip. The detailed pedigree data available in this cohort positioned us to estimate heritability (h2) of circulating amine metabolites. We estimated h2 and conducted genome wide associations with metabolite levels. Analyses were conducted using our mixed models analysis for pedigrees and populations (MMAP) software: http://edn.som.umaryland.edu/mmap/index.php Residual h2 was calculated for metabolites adjusting for age and sex. Twenty metabolites were heritable (p 0.7 ($p<0.01$). Genome-wide association analysis identified several amines, including DL-3-aminoisobutyric acid, phenylalanine, and hydroxylysine with highly significant associations, some of which replicated on the exome chip array. For example, on GWAS, baseline DL-3-aminoisobutyric acid levels were associated with a chromosome 5 variants in AGXT2, a gene which encodes an aminotransferase that catalyzes conversion of glyoxylate to glycine. Exome chip data found aminoisobutyric acid levels were associated with SNPs in AGXT2, including rs16899974 and rs37369. As well, on GWAS, pre-intervention phenylalanine levels were associated with variants in the chromosome 12 PAH gene, which encodes phenylalanine hydroxylase. Exome chip data similarly mapped pre-intervention phenylalanine levels to a PAH variant, rs5030849, known to be related to a mild form of phenylketonuria. This study's approach, which harnesses the power of combining GWAS techniques and LCECA measures of serum metabolites, highlights the power of integrating genomics and metabolomics to enhance knowledge of the mechanisms underlying baseline metabolite levels. Our results shed light on the synergies of cross disciplinary collaboration which result from combining the efforts of scientists from the Metabolomics Network with investigators from the Pharmacogenomics Research Network. This analysis underscores the genetic contribution to amine metabolite levels and identifies genome-wide significant variants associated with amine metabolite levels.

oral 057	15 min	pharmacometabolomics	Drew Neavin * PhD student

Pharmacometabolomics reveals SOD2 as a tryptophan metabolite ratio regulator associated with selective serotonin reuptake inhibitor response in major depressive disorder

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Major depressive disorder (MDD) is a debilitating disease that afflicts 20 million Americans annually. The first line class of medication for MDD is selective serotonin reuptake inhibitors (SSRIs). However, at least 30% of patients

with MDD do not respond to SSRIs. The balance of tryptophan metabolism between the serotonin and kynurenine pathways is hypothesized to play a role in MDD pathophysiology. Therefore, there may be a relationship between tryptophan metabolism balance and individual SSRI response. We set out to identify genes associated with tryptophan pathway imbalance that may influence SSRI response. Eight hundred MDD patients were treated with the SSRIs, citalopram or escitalopram. Blood was drawn at baseline, 4 weeks and 8 weeks for SNP genotyping, blood drug level assays and liquid chromatography electrochemical array (LCECA) metabolomic concentrations. Changes in serotonin and kynurenine pathway metabolite ratios between baseline and 4 weeks or baseline and 8 weeks were calculated and correlated with patient response. Genome-wide association studies (GWAS) were performed to correlate single nucleotide polymorphisms (SNPs) with changes in metabolite ratios. mRNA knockdown and reverse transcription-quantitative PCR (RT-qPCR) were used to functionally validate the role of the major gene identified during the GWAS. Four metabolite ratios were correlated with clinical SSRI response: serotonin/kynurenine, serotonin/tryptophan, serotonin/5-hydroxyindole acetic acid, and serotonin/3-hydroxykynurenine. SNPs across, 3', and 5' of the superoxide dismutase 2 (SOD2) gene were highly associated with 4- and 8-week change in all of these same metabolite ratios. For example, the top SNPs (none of which were genome-wide significant) for change at 8 weeks were: serotonin/kynurenine, rs199968457, p = 1.29E-06 serotonin/tryptophan, rs199968457, p = 3.01E-07 serotonin/5-hydroxyindole acetic acid, rs199968457, p = 1.59E-06 serotonin/3-hydroxykynureinine, rs2842984, p = 1.83E-07 Some of these SNPs associated with tryptophan metabolite ratio changes were eQTLs, ie they were associated with SOD2 transcription. SOD2, a mitochondrial enzyme, which reduces reactive oxygen species by catalyzing the conversion of superoxide into hydrogen peroxide, has not previously been associated with the tryptophan pathway. However, functional validation by SOD2 knockdown in glioma cells revealed increased expression of MAO A (4.7 + 0.58 fold, p = 4.3E-06) and IDO1 (5.8 + 0.78 fold, p = 1.5E-04). MAO A is mitochondrial enzyme that catalyzes serotonin catabolism, and IDO1 is the rate-limiting enzyme that catalyzes the synthesis of kynurenine from tryptophan. These results suggest that SOD2 may be important in tryptophan metabolism balance and SSRI response, perhaps in part by affecting MAO A and IDO1 expression. Using metabolite ratios for GWAS is a novel approach which revealed that SOD2 may be a regulator of tryptophan metabolism.

oral 058	15 min	pharmacometabolomics	Douglas Kell

A human metabolic network reconstruction reveals the metabolite-likeness of approved pharmaceutical drugs: a 'rule of 0.5'

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A considerable amount of evidence, summarised in [1-7], indicates that pharmaceutical drugs and xenobiotics do not normally 'float' or 'leak' across biomembranes but require protein transporters in order to enter and exit cells. These transporters are there not for the benefit of Pharma companies but are part of intermediary metabolism. A recent curated reconstruction of the human metabolic network, of which fully one third of the steps involve transporters, identifies those metabolites [8,9]. The principle of molecular similarity implies that if drugs hitchhike on these transporters they should have structural similarities to their substrates (i.e. endogenous metabolites) and allows us to ask about the extent to which they do [10]. Using standard methods of cheminformatics for encoding the structures of marketed drugs and endogenous metabolites we compared (using KNIME workflows; http://www.knime.org) their structural likenesses using the Tanimoto similarity (TS a metric that lies between 0 and 1). Using the so-called MACCS encoding it was found that more than 90% of marketed drugs had a TS of greater than 0.5 to at least one endogenous metabolite. "While this does not mean, of course, that a molecule obeying the rule is likely to become a marketed drug for humans, it does mean that a molecule that fails to obey the rule is statistically most unlikely to do so" [10]. This provides a valuable, mechanistically based filter. [1] Kell, D. B., Dobson, P. D. & Oliver, S. G. (2011). Pharmaceutical drug transport: the issues and the implications that it is essentially carrier-mediated only. Drug Disc Today 16, 704-714. [2] Kell, D. B., Dobson, P. D., Bilsland, E. & Oliver, S. G. (2013). The promiscuous binding of pharmaceutical drugs and their transporter-mediated uptake into cells: what we (need to) know and how we can do so. Drug Disc Today 18, 218-239. [3] Kell, D. B. (2013). Finding novel pharmaceuticals in the systems biology era using multiple effective drug targets, phenotypic screening, and knowledge of transporters: where drug discovery went wrong and how to fix it. FEBS J 280, 5957-5980. [4] Kell, D. B. & Oliver, S. G. (2014). How drugs get into cells: tested and testable predictions to help discriminate between transporter-mediated uptake and lipoidal bilayer diffusion. Front Pharmacol 5, 231. [5] Kell, D. B. & Goodacre, R. (2014). Metabolomics and systems pharmacology: why and how to model the human metabolic network for drug discovery. Drug Disc Today 19, 171-182. [6] Kell, D. B. (2015). What would be the observable consequences if phospholipid bilayer diffusion of drugs into cells is negligible?

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oral 059	30 min	imaging and MS-integration methods	Alfredo J. Ibanez

Benefits and Pitfalls of a single-cell level metabolomics technique: 5 years of Microarrays for Mass Spectrometry

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In 2010, the Zenobi group introduced for the first time a novel technology called MicroArrays for Mass Spectrometry (MAMS). This technology is capable of boosting the sensitivity of traditional matrix-assisted laser desorption/ionization mass spectrometers (MALDI MS) to achieve single-cell level analysis. Since then, MAMS has been used to dissect the differences between co-existing phenotypes in vast cell populations. Nevertheless in order to better monitor the cell-to-cell heterogeneity, we needed to keep improving the degree of robustness associated with the MAMS-based measurements. Here, we present a summary of the know-how accumulated in the past five years. In particular, we will focus on those technical/analytical developments that made the MAMS technology become a more robust single-cell technique. MAMS are microarrays that uses a combination of hydrophilic reservoirs surrounded by an omniphobic surface for massively parallel, automated sample spotting. MAMS are produced from commercial transparent indium-tin oxide coated cover glass chips (20 mm x 20 mm x 0.16 mm, SPI Supplies, Unterfoehring, Germany). The slides are spin-coated (SuSos, Duebendorf, Switzerland) with a 1 µm thick polysilazane coating (CAG 37, marketed by Clariant, Frankfurt am Main, Germany). This polysilazane layer is structured (EMPA, Thun, Switzerland) in an array of circular recipients using a projection laser ablation system equipped with an excimer laser (Exitech Ltd., Oxford, UK). For the past 5 years, we have been implementing different approaches for the fabrication of the MAMS substrates. One of the latest methods we tried, projection laser ablation, allowed us to further reduce the technical variability associated with their production. In addition, using the know-how associated with population-level Metabolomics and DNA/RNA microarray applications, we simultaneously improved the cell-handling protocols as well as the data processing of our single-cell level mass spectrometry-based measurements. Finally, when these overall improvements are combined with a set of simple rules, we not only bestow lower levels of technical/analytical variability to our single-cell level mass spectrometry measurements (i.e., increased robustness), but we are now able to better identify the biologically significant signals. With this increased confidence, we applied the MAMS-based analytical platform to monitor at the single-cell level a clonal population of the model organism Saccharomyces cerevisiae, and characterized the observed cell-to-cell heterogeneity within the yeast population in terms of the different levels of glycolytic intermediates. MAMS is able to calculate the cell-to-cell differences in glycolytic intermediates, an insight that is not accessible with other techniques.

oral 060	15 min	imaging and MS-integration methods	Kerem Bingol

The SUMMIT MS/NMR Method for the Rapid Identification of New Metabolites in Complex Mixtures

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Many of the signals found in NMR and MS spectra of complex metabolite mixtures belong to molecules whose identification is notably hard. Traditionally, identification of unknown metabolites requires their isolation through time-

consuming purification from complex mixtures by using separation techniques, such as chromatography, followed by extensive characterization by combining NMR, MS, X-ray, and other techniques. Here, we introduce an alternative metabolite identification strategy of complex mixtures by combining MS with NMR in a novel way. Since the method does not require purification, it is suitable for high-throughput identification of new molecules in crude metabolite extracts. We term this approach SUMMIT MS/NMR for “Structures of Unknown Metabolomic Mixture components by MS/NMR” (Bingol, K. et al. Anal. Chem. 2015, DOI: 10.1021/ac504633z). The workflow of SUMMIT MS/NMR is the following: for a sample of a complex metabolite mixture of unknown composition, the high-resolution mass spectrum is determined and accurate masses are converted to molecular formulas. For each molecular formula, all possible structures (“structural manifold”) are generated. The NMR spectrum (chemical shifts) of each structure is then predicted. Meanwhile, the experimental NMR spectrum is determined for the same mixture and deconvoluted into the NMR spectra of individual components. The NMR spectrum of each component is compared to the predicted NMR spectra of the structural manifold and the structures are rank-ordered according to their agreement to identify those molecular structures that are most consistent with all available NMR and MS data. After successful testing on a model mixture, SUMMIT MS/NMR was applied to an E. coli cell extract, which was injected into a Q-TOF mass spectrometer. From the resulting MS spectrum 56 molecular formulas could be extracted. For each molecular formula all feasible structures were determined using ChemSpider, resulting in a total structural manifold of 13,872 structures. 13C-1H HSQC spectrum of each of these structures was predicted by using MestReNova software. For the same E. coli sample, 13C-1H HSQC, 1H-1H TOCSY, 13C-1H HSQC-TOCSY and 13C-1H HMBC spectra were acquired. 13C-1H HSQC was then deconvoluted into subspectra belonging to individual mixture components by using connectivity information derived from TOCSY and HMBC. The chemical shift list of each HSQC subspectra was quantitatively compared one-by-one against the peak lists predicted for each manifold structures using a scoring function that is analogous to the one used for the querying of HSQC spectra against COLMAR webserver. Aspartate, alanine, betaine, GABA, glutamine, arginine, lysine, methionine, N-acetylputrescine, spermidine, tyrosine, threonine, uracil, and nicotinate were returned as the top hits among the 13,872 structures. Isoleucine and phenylalanine were returned as second best hits, while adenosine, glutamate, leucine, and valine were returned as third-best hits among all 13,872 structures. In most cases, false positive structures, which were returned as best hits, can be easily excluded by adding additional constraints from other NMR experiments such as 1H-1H TOCSY, which is already available in the collected set of data. These results clearly demonstrate the power of NMR chemical shift information as an effective filter to identify the correct structures among the large structural manifold belonging to MS-derived molecular formulas. SUMMIT MS/NMR neither requires purification nor the use of NMR and MS metabolite databases and it is applicable to a wide range of complex metabolite mixtures ranging from biomedicine, biology, to food sciences. SUMMIT MS/NMR is a new metabolite identification strategy of unknown molecules in complex mixtures without the need of spectroscopic databases.

oral 061	15 min	imaging and MS-integration methods	Alison McGuigan

TRACER: A novel strategy for assessing metabolomic signatures from cells in 3D heterogeneous microenvironments

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Local microenvironment is a critical component in determining cellular metabolism. Tumours are heterogeneous structures in which the local microenvironment varies extensively. Assessing variations in metabolic behaviour within a solid tumour presents an experimental challenge however: metabolite turnover is rapid compared to changes in mRNA and proteins and consequently, profiling intracellular metabolites requires rapid quenching of metabolic activity prior to analysis. Studies on metabolic adaptations in tumour cells are therefore currently limited to homogeneous 2D or suspension cultures that facilitate rapid collection of metabolite samples for analysis. Alternative experimental models that better recapitulate the 3D microenvironment of a tumour are available, but none enable rapid collection of cells from defined locations to spatially correlate different microenvironments to snap shot metabolic signatures. We have developed an approach to assemble 3D engineered tumours by rolling thin (30 micron) tumour cell-impregnated scaffolds on an oxygen impermeable core. In our Tumour Roll for Analysis of Cell Response (TRACER), cells at different depths within the roll experience different oxygen and nutrient levels mimicking the variation seen in tumours

at progressively further distances from a blood vessel. Our system can be rapidly (<1s) disassembled for analysis by unrolling. The TRACER geometry facilitates mapping of cell location along the length of the 2D strip to their location in the 3D tumour to facilitate separation of populations of cells from different microenvironments. Furthermore, cell isolation can be achieved rapidly enabling assessment of metabolic profiles. We have generated TRACERs using a range of cancer cell lines. We have shown that oxygen gradients develop within the TRACER between 6 and 48h of culture resulting in graded cell death in progressively deeper layers by 72h. We have validated the physiological relevance of the TRACER model: cells in TRACER faithfully replicate known oxygen sensitivity for hypoxia induced genes. HIF target genes were induced after mild hypoxia (middle layers) whereas unfolded protein response target genes were induced by more severe hypoxia (deeper layers). Furthermore, cells in the inner hypoxic layers of the TRACER show greater therapy resistance in response to radiation as is seen in tumours in vivo. Using the TRACER system combined with liquid chromatography tandem mass spectrometry we mapped cellular metabolism in 3D. Both unsupervised hierarchical clustering and principal component analysis revealed a clear separation of samples consistent with TRACER layers and oxygen levels, indicating that the different environmental conditions associated with each layer induce distinct metabolic signatures. In addition, we identified metabolites whose intensity across the TRACER layers correlated significantly with levels of hypoxia quantified by EF5 binding. Of the 88 metabolites analyzed, 23 showed significant correlations with EF5 levels including metabolites associated with glucose metabolism (lactate, hexose, glycerate, GA3P and alanine) and mitochondrial function (succinate), consistent with a glycolytic switch under hypoxia. We also observed changes in several urea cycle metabolites, including a decrease in arginine and an accumulation of argininosuccinate (ASA) in the hypoxic layers, consistent with known hypoxic inhibition of the urea cycle enzyme Argininosuccinate Lyase. Furthermore, 2-hydroxyglutarate, a metabolite recognized for its oncogenic properties, accumulated in the innermost layers of the TRACER and decreased levels of reduced glutathione were observed in inner layers, consistent with increased oxidative stress under hypoxia. The TRACER method facilitates, for the first time, spatial profiling of cellular metabolism in 3D heterogenous tissues

oral 062	15 min	imaging and MS-integration methods	Basil Nikolau

Mass Spectrometric Imaging of Plant Metabolites

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The research aims to develop and apply mass spectrometric imaging (MSI) technologies and spatially map metabolite distributions within plant tissues. Such imaging technology is of particular importance in multicellular organisms, such as plants, in order to identify and characterize cooperative and antagonistic genetic interactions, and environmental modifiers that asymmetrically regulate the complexity of plant metabolism. We are using the MSI techniques to address specific biological questions concerning the differential distribution of metabolites among diverse cell-types, and dissect metabolic regulatory processes. Interactions among analytical chemists and biologists is providing a context for developing specific metabolite imaging capabilities to better understand specific plant metabolic processes. We are adapting MALDI-based and atmospheric pressure laser desorption ionization (APLDI) methods to image the distribution of metabolites at high spatial resolution. With MALDI the current state of our technology provides 5-µm spatial resolution, whereas with APLDI the resolution is approximately 50-100-µm. The research is focused on four significant metabolic systems: 1) germinating maize seeds; 2) C4 photosynthesis cells of maize leaves; 3) Biosynthesis of maize cuticular lipids; and 4) polyketide and flavonoid natural products in secretory structure (translucent glands or cavities, black nodules and secretory canals) of Hypericum leaves. These systems were specifically targeted because they offer the ability to decipher the asymmetric distribution of metabolites among discreet cellular-sized structures, and such data is highly revealing of the underlying regulatory mechanism(s) that control these complex metabolic processes. Integrating high-spatial resolution MSI data with global genome expression profiling (either at the level of the transcriptome or proteome) is providing testable hypothesis concerning gene-functions. Validating these hypotheses via functional genomics analysis is revealing more accurate predictive understanding of the mechanisms that multicellular organisms use to regulate metabolic processes. We have developed and applied novel mass spectrometric imaging technologies to reveal the spatial organization of metabolic processes.

oral 063	15 min	imaging and MS-integration methods	Trent Northen

Kinetic Mass Spectrometry Imaging

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Traditional metabolic profiling methods provide an unbiased analysis of cellular metabolism and have found a diverse range of applications. While these approaches maximize the number of metabolites detected, they depend on solvent extraction methods that result in a loss of spatial information and ignoring much of the inherent complexity of biological systems. Therefore it is critical to complement these profiling methods with small molecule imaging approaches. This talk will present new technologies for mass spectrometry based imaging to study dynamic metabolic turnover of lipids within the 3D tumor microenvironment and within microbial co-culture biofilms. Administration of 5-10% deuterated water (2H2O) provides a mechanism for the dynamic biosynthetic incorporation of deuterium into lipids and measurement of de novo lipogenesis. For tumor imaging, 2H2O delivered as a intraperitoneal bolus dose and via drinking water whereas in biofilm imaging the 2H2O is added directly to agarose plates. Newly synthesized lipid becomes labeled with deuterium in an amount proportional to the atom% deuterium enrichment of cellular water and the number of hydrogens that can be biochemically incorporated into that lipid. Mass spectrometry imaging is then performed using an ABI/Sciex 5800 TOF/TOF mass spectrometer. We find that kinetic mass spectrometry imaging (kMSI) addresses several major challenges to mass spectrometry imaging. First it localizes metabolic flux which has proven extremely valuable in LC-MS methods. Second, since the data analysis is based on comparison of unlabeled to labeled metabolite pools, it controls for matrix effects that can often confound traditional MSI methods. kMSI, applied to a tumor section as part of a low-dose ionizing radiation study, enabled correlation of new synthesis of specific lipids that discriminated high grade versus low grade tumor regions; region grades were confirmed by correlative analysis of traditional histopathology stains. Specifically, this approach revealed where new synthesis of each lipid occurred and the ratio of newly synthesized vs. pre-existing lipid. Variations in de novo lipogenesis and lipid composition were mapped to spatially-distinct regions, suggesting altered metabolism specific to cell subpopulations comprising each region. Extension of this approach to analysis of microbial interactions reveals differential spatially defined metabolite turnover resulting from microbial interactions. kMSI enables the spatially defined determination of lipid composition and biosynthesis rates within complex biological matrices.

oral 064	30 min	pathways and microbial metabolism	Uwe Sauer

Near real-timing metabolome profiling reveals dynamics and regulation of the starvation to growth transition in E. coli

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Metabolism is often the first responding network to environmental changes and metabolite dynamics are key to understand these cellular responses. Monitoring metabolome changes is experimentally tedious and demanding, hence dynamic data on time-scales from seconds to hours are scarce. Here, we developed near real-time metabolome profiling by directly injecting living bacteria and yeast into TOF mass spectrometer based on our previously reported flow injection TOF method (Fuhrer et al. 2011 Anal Chem 83: 7074; Sevin & Sauer 2014, Nature Chem Biol 10: 266). Near real-time profiling enabling automated monitoring of about 300 compounds in 15-30 second cycles over several hours. By applying the method to the transition from carbon starvation induced stationary phase to resumption of growth of Escherichia coli, we found unexpected accumulation of energetically costly biomass metabolites including certain amino acids and purine metabolites during starvation that were rapidly utilized upon growth resumption. By combining, near real-time metabolome profiling with modeling and inhibitor experiments, we demonstrated switch-like feedback inhibition in amino acid biosynthesis. Instead of end-product inhibition, preferential use of the metabolically cheaper 1-step salvaging pathway over the costly 10-step de novo purine biosynthesis during growth resumption was controlled by substrate availability. We present to our knowledge the first near real-time analysis of a larger metabolome by mass spectrometry.

oral 065	15 min	pathways and microbial metabolism	Andreas Kühne * PhD student

Identification of sites and timings of metabolic regulations from metabolite profiling data using probabilistic graphical modelling

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Reciprocal crosstalk of metabolism and cellular signaling pathways was recently shown to play a crucial role in cellular decision-making. This motivated several metabolomics studies on various cellular processes in different organism, which lead to a vast availability of metabolomics data sets. To date, one of the major challenges in metabolomics remains the interpretation of metabolite levels, which result from the kinetic properties of enzymes and complex molecular interactions. Current computational methods rely heavily on pre-defined metabolite sets and stiff definitions of metabolic pathways and thus can lack flexibility to identify important metabolic adaptations. Here, we present a novel probabilistic graphical modelling approach that aims at identifying motifs of metabolic adaptations merely from metabolite profiling data. The model is drafted as a Markov random field (MRF) with hidden states representing metabolites, observables that integrate measured metabolite levels, and dependencies between hidden states based on the underlying biochemical reaction network. Specifically, this model assumes that hidden states emit a certain metabolic observation with a label specific probability and that labels of hidden states are dependent on the labels of the neighboring states. Optimizing the hidden state label distribution to fit measured metabolite profiles enables to identify modules of neighboring states with the same label. Transitions between two different modules indicate possible sites of metabolic regulation. In contrast to state-of-the-art methods, our approach is independent of artificial pathway definitions and deals seamlessly with noisy and incomplete measurements. Given comparative metabolomics data and a genome scale metabolic network, our algorithm automatically identifies sites of metabolic regulation and ranks them according to their significance. Application on metabolomics data from Escherichia coli enzyme and transcription factor knock-out mutants demonstrated that our method outperforms alternative approaches in identifying removed enzymes. Additionally, we identified potential novel direct or indirect targets of the transcription factor purM. Moreover, the approach can be extended for time course measurements by adding the previously described MRFs for individual time windows and introducing dependencies for hidden states of sequential windows. Preliminary results demonstrate that performing the inference on this temporal extension enables to investigate the change in activity of certain metabolic modules as well as the timings of those changes to improve classification of time course data. Thus, this approach represents a novel and powerful method that can be broadly applied for the extraction of non-trivial regulation motifs from metabolite profiling data. Novel and broadly applicable method to identify sites and timings of metabolic regulation using large-scale metabolomics data and metabolic networks.

oral 066	15 min	pathways and microbial metabolism	Eun Ju Yun * Early Career Scientist

The first elucidation of the novel metabolic pathway of 3,6-anhydro-L-galactose by integrative analyses of metabolome and transcriptome

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Recently, marine macroalgae have been investigated as renewable sources of carbohydrates for producing fuels and chemicals. The most abundant carbohydrate polymer of red macroalgae is agarose, which is composed of D-galactose and 3,6-anhydro-L-galactose (AHG) with alternate α-1,3 and β-1,4 linkages. Although agarose is considered a valuable source of fermentable sugar, its major sugar, AHG, is known to be not fermentable by industrial microorganisms. Due to the high abundance of AHG in red macroalgal biomass, the inability of fermenting AHG impedes the overall utilization red macroalgal biomass as the biomass feedstock. For the first time, we have discovered the novel metabolic pathway of AHG using a marine microorganism by integrating metabolome and transcriptome analyses. Using the Vibrio sp. strain EJY3 strain as a model organism to study the AHG metabolism in marine bacteria, we performed metabolite and transcriptomic analyses to identify key metabolic intermediates and the corresponding enzymes that are thought to be involved in catabolizing AHG. Testing the reactivity of the recombinant

proteins validated the molecular functions of predicted genes. Furthermore, to examine the in vivo reactivity of the newly discovered metabolic route for AHG, we introduced their genes from EJY3 into Escherichia coli and tested the growth of the recombinant E. coli on AHG as a sole carbon source. The integrative analyses of metabolome and transcriptome of Vibrio sp., a marine bacterium capable of catabolizing AHG as a sole carbon source, revealed two key metabolic intermediates of AHG, 3,6-anhydrogalactonate (AHGA) and 2-keto-3-deoxygalactonate and their corresponding genes that were verified both in vitro and in vivo. Oxidation by an NADP+-dependent AHG dehydrogenase and isomerization by an AHGA cycloisomerase are the two key AHG metabolic processes. This newly discovered metabolic route was verified in vivo by demonstrating the growth of E. coli harbouring the genes of these two enzymes on AHG as a sole carbon source. Also, the introduction of only these two enzymes into an ethanologenic E. coli strain increased the ethanol production in E. coli by fermenting both AHG and galactose in an agarose hydrolysate. This is the first report on the metabolic pathway discovery of AHG, a rare sugar, in a living organism.

oral 067	15 min	pathways and microbial metabolism	Sarah Schatschneider * Early Career Scientist

Metabolic Pathway Profiling of Clostridium autoethanogenum for Sustainable Biofuel Production

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Climate change and limited supply of fossil energy have forced the world to reduce greenhouse gas emissions and find sustainable ways for the production of energy and chemical commodities. The importance of this is reflected in the Conference Satellite Meeting “Metabolomics for a Low Carbon Society”. Acetogenic Clostridium species are able to use waste gases in the form of CO or CO2 through anaerobic gas fermentation via operation of the Wood-Ljungdahl pathway. Carbon monoxide or CO2 and hydrogen can be converted into ethanol and other useful chemical intermediates. Thus, gas fermentation represents a promising platform for the sustainable production of biofuels and chemicals. We investigate key metabolic pathways of C.autoethanogenum to overcome bottle-necks for conversion of gases to chemical intermediates. Targeted metabolite measurements using liquid chromatography-mass spectrometry were performed for analysis of the Wood-Ljungdahl pathway and central carbohydrate metabolism. Metabolites of interest were analysed by hydrophilic interaction liquid chromatography (ZIC-pHILIC) coupled with high resolution mass spectrometry (Orbitrap Exactive). We used a commercially available universally 13C-labelled Spirulina cell powder to enable isotope dilution mass spectrometry (IDMS) for the identification and quantification of intracellular metabolites. Identity of metabolites was confirmed by HRMS and by tandem MS measurements. Volatile fermentation products and intermediates (not measurable by LC-MS) including acetic acid, ethanol, 2,3-butanediol and isobutanol were measured in the extracellular growth medium using gas chromatography-mass spectrometry (GC-MS). To understand and optimise key steps of C. autoethanogenum in gas fermentation we investigated the metabolic pathways of this organism in more detail. Clostridium autoethanogenum DSM10061 was grown continuously in a 3L bioreactor on carbon monoxide as the major carbon source and samples were taken in different phases of gas fermentation. Concentrations of metabolites were calculated by supplementing known amounts of U-13C-Spirulina cell extracts to extraction solution as well as to standard compounds. In total, we analysed 64 polar metabolites in C. autoethanogenum by comparison of retention time and accurate mass determination < 2ppm. The list of compounds analysed includes amino acids, carboxylic acids, sugar phosphates, purines, and pyrimidines. For the visualisation of metabolic data we established a metabolic network reconstruction of C. autoethanogenum metabolism based on the published, genome sequence of this organism. A draft metabolic network was established in systems biology mark-up language which consists of 188 genes, 273 enzymatic reactions and 291 metabolites. In addition to its suitability for data visualisation, the metabolic network will be basis for metabolic network modelling for predictions of beneficial metabolic alterations. (Acknowledgements: This work is funded by LanzaTech, BBSRC and SBRC Nottingham) Application of commercial affordable U-13C Spirulina extract for quantification of metabolites, large-scale metabolic network of C. autoethanogenum for metabolic engineering

oral 068	15 min	pathways and microbial metabolism	Yifan Xu * Early Career Scientist

Toward a comprehensive understanding of cellular metabolism

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Microbial metabolism and its regulation are relevant both for metabolic engineering and as a model system for metabolic diseases such as cancer and diabetes. Although microbial metabolism has been extensively studied, some pathways remain ill-defined and, even for the best defined pathways, their regulation remains incompletely understood. New tools that allow direct and systematic measurement of metabolites and their fluxes by liquid chromatography-mass spectrometry (LC-MS based metabolomics), hold the potential to address these limitations. Here we show examples initiated by LC-MS based metabolomics to address the most important and challenging metabolic puzzles. A combination of LC-MS based metabolomics (UPLC-Orbitrap), genetics, chemical genetics, biochemistry and physiological studies was used to investigate gaps in microbial metabolism and its physiological regulation. We also optimized our LC-MS methods and summarized commonly overlooked misannotation and misquantitation issues caused by in-source fragmentation (Xu et. al. Analytical Chemistry, 2015). Here we show a number of recently highlighted studies initiated by metabolomics: 1. Characterizing a very challenging yet important autophagy pathway: Previously poorly characterized enzymes work in concert to degrade nucleotides in yeast, and this pathway is essential for yeast's survival in nutrient starvation and stress (Xu et. al. Molecular Systems Biology, 2013). 2. Even in glycolysis – the most well studied metabolic pathway – metabolic regulation was poorly understood: Previously under-appreciated regulation – allosteric activation by FBP – controls glycolytic efflux in both E. coli and yeast in changing nutrient environment. Previously emphasized regulation including phosphorylation was irrelevant (Xu et. al. Nature Chemical Biology, 2012; Xu et. al. Molecular Cell, 2012). 3. Yeast survives in oxidative stress by running a noncanonical central metabolism: Instead of using glucose and glycolysis, yeast utilizes autophagy and a "Pento-Hexose" cycle to regenerate antioxidant (Xu et. al. In preparation, 2015). Studies of this type, by systematically clarifying enzyme functions and physiologically studying pathway regulation, hold the potential for leading to more predictable metabolic engineering and more comprehensive understanding of cellular metabolism. Metabolic mapping of autophagy pathways; Physiologically relevant regulation in glycolysis; Noncanonical central metabolism in oxidative stress; Overlooked metabolite misannotations

oral 069	30 min	compound ID	Emma Schymanski * Early Career Scientist

Exploring Cheminformatic Approaches for Compound Identification using High Resolution Tandem Mass Spectrometry Data

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Cheminformatic methods have opened up a world of possibilities for higher throughput data exploration in recent years. From predicting metabolism, looking for "suspected" substances, comparing water in different continents through to querying the world wide web, cheminformatics is here to stay. This talk will showcase the different approaches for compound identification used at Eawag to answer a wide range of research questions, following the three-pronged attack of target (known), suspect and non-target (unknown) screening. Target and suspect screening was performed with enviMass. High resolution isotope, adduct and homologue information was extracted using the R package non-target; MS/MS information was extracted using RMassBank. MOLGEN-MS/MS was used to calculate molecular formulas, the fit was visualised with enviPat. MetFrag and MetFusion were used to retrieve structures from ChemSpider and PubChem; the latter also retrieving spectral information from MassBank. RMassBank was used to create recalibrated, noise-reduced, subformula-annotated MS/MS spectra for MassBank querying CTS for compound information, to form test datasets for workflow evaluation. Suspected metabolites were calculated using the Eawag Pathway Prediction System (Eawag-PPS); structure generation was performed with MOLGEN. Most spectra were acquired on high resolution Orbitrap mass spectrometers coupled to a reverse phase HPLC. Despite the availability of several hundred target compounds, peak inventories of wastewater revealed that the target compounds comprise less than 2 % of the total number of peaks detected in typical samples (>15,000 peaks, after blank subtraction). This strongly supports the need for higher throughput suspect and non-target screening methods in MS/MS-based identification. While a median rank of 1 can be achieved with MetFusion on reference compounds for high resolution data, this does not hold when no similar compounds are present in MassBank. The results of CASMI 2013 shows that

the ranking power is not sufficient to expect the correct structure in the top 10 for large queries (>1000 candidates), often achieved with PubChem or ChemSpider. Thus alternative strategies are needed. The example of benzotriazole metabolism in activated sludge shows how suspect (predicting metabolism) and non-target screening (following time trends) were used to characterize the main metabolites of the benzotriazoles. As the identification was performed using structure generation, this formed an ideal base dataset to expand MetFrag and MetFusion to deal with substructure restrictions during compound database retrieval. The detection of isotopic fine structure (especially 34S) in wastewater samples led to the development of “suspected homologue” screening techniques. Literature data, homologue screening, chromatography and mass spectral evidence was used to link members of homologous series which could not otherwise be identified (absence from open compound databases; scarcity of reference standards; insufficient intensity for MS/MS for most members of the series). Evidence of these compounds has now been found in samples across Europe and in Canada. Finally, the importance of effective suspect screening, data sharing and communicating the identification confidence, along with common pitfalls and future perspectives, will be highlighted using data from a collaborative non-target screening trial run on a sample from the River Danube, with 18 participating institutes. Demonstration of the current possibilities and future needs in applying cheminformatics to compound identification using database approaches

oral 070	15 min	compound ID	Tobias Kind

Advances in in-silico prediction methods of mass spectra for improved metabolite identifications

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We present an overview about the current state of the art of in-silico generation of mass spectra. We discuss heuristic rule-based approaches, probabilistic machine learning techniques and first-principle methods based on quantum mechanics and quantum chemistry. We show results from three different approaches including two independent LC-MS/MS related modelling approaches of tandem mass spectra as well as GC-MS related modelling of electron ionization mass spectra. Advantages and disadvantages are objectively discussed utilizing external validation approaches and library scoring. The application of such computer generated libraries is shown for metabolites involved in human diseases such as diabetes and for plant, algal and animal based research. (1) LipidBlast templates were employed to heuristically model collision induced dissociation (CID) tandem mass spectra. Reference compounds were measured on a QTOF instrument under different CID voltages. (2) The Competitive Fragmentation Modeling software (CFM-ID) (Allen/Wishart, University of Alberta) uses a stochastic, homogeneous Markov process to generate in-silico MS/MS mass spectra for different CID voltages. We utilized 6000 MS/MS spectra from MassBank and NIST to investigate match scores and false-positive and false-negative statistics. (3) The software QCEIMS (Grimme, University of Bonn) was utilized to generate 70V electron ionization spectra of compounds. QCEIMS uses quantum mechanics and molecular dynamics gas phase reaction modelling including direct functional theory (DFT), dispersion corrected models (DFT-D3) and semiempirical methods. We discuss results from three different in-silico approaches such as the heuristic LipidBlast, the probabilistic CFM-ID and the quantum chemistry based QCEIMS. (1) We used the LipidBlast templates to develop and validate a library for a new class of fatty acid esters of hydroxy fatty acids (FAHFAs) that were recently discovered by (Yore/Kahn, Harvard University) for treating type 2 diabetes and reducing inflammation. We modeled the 1080 most probable lipids of this new class, including Q-TOF optimized voltages for 10V, 20V, 40V under collision induced dissociation (CID). Validation was performed with reference compounds and reversed phase liquid chromatography. (2) The CFM-ID software was used to generate 6000 MS/MS spectra covering 2000 metabolites. We compared the generated 10V, 20V, 40V CID QTOF spectra against high-quality reference spectra from MassBank and NIST. For compounds that cover the same compound diversity space as the development set 70% of the metabolites are correctly identified as first hit. We investigated true-positive and false-positive rates by increasing the diversity of the dataset and performed statistical investigations using dot-product, reverse-dot-product and probability based MS/MS scoring. Increasing the compound diversity the true identification rates expectedly drop to lower values. Interestingly the probability based MS/MS matching outperformed the vector-product based scoring for these in-silico spectra. (3) QCEIMS is the first quantum chemical generator of electron ionization mass spectra. We discuss generated mass spectra based on calculated ionization energies and molecular reaction trajectories. The reaction trajectories can be used to visualize and understanding gas-phase fragmentations. Fragment structures and their peak abundances allow for direct matching and validation

against large mass spectral databases. It is currently not possible to synthesize authentic reference standards or perform de-novo structure elucidation for millions of existing and virtual compounds. In-silico generated mass spectra prove to be a fast-track for faster and reliable compound annotations. In-silico libraries allow reference spectra creation without chemical standards; hence they can also cover large spaces of the unknown metabolome.

oral 071	15 min	compound ID	Sebastian Böcker

Searching molecular structure databases with tandem mass spectra using CSI: FingerID

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Structural elucidation of small molecules from mass spectrometry data remains a challenging problem, in particular for compounds that cannot be found in any spectral library. Available spectral libraries for tandem MS data of small compounds are orders of magnitude smaller than molecular structure databases such as PubChem. Searching tandem mass spectra directly in molecular structure databases is therefor considered a powerful tool for assisting an expert in the structural elucidation of a compound. Several strategies have been proposed during the last years, including simulation of mass spectra from molecular structure, combinatorial fragmentation, and prediction of molecular fingerprints from the mass spectrometry data. I will present CSI (Compound Structure Identification):FingerID for searching a molecular structure database using MS/MS data. The method combines computation and comparison of fragmentation trees with machine learning techniques for the prediction of molecular properties of the unknown compound. Our method shows significantly increased identification rates compared to all existing state-of-the-art methods for the problem. In contrast, fragmentation trees have been introduced as a means of analyzing tandem MS data without the need of any (structural or spectral) database, and for determining the molecular formula of an unknown compound. Using a novel scoring based on Maximum a Posteori Estimation, we were able to significantly increase the number of compounds for which the correct molecular formula could be detected. I will present our current results for searching molecular structure databases with tandem MS data. We have trained and evaluated our method on a dataset with 5,923 compounds using cross-validation. Searching the complete PubChem database, CSI:FingerID currently reaches 34.4% correct identifications. This is a 2.5-fold increase in correct identifications compared to existing methods for this task (FingerID, CFM-ID, MAGMa, MetFrag,MIDAS) which reach less than 13.9% correct identifications on this dataset. We achieve 63.5% correct identifications in the top 5 output, for which competing methods reach less than 36.2%. Our method reaches an identification rate of 50% for the top 3, whereas any competing method requires at least to consider the top 14 for this identification rate.Adding about 400 compounds to the training data increases our identification rate by roughly one percentage point. CSI:FingerID reaches 39.5% correct identifications on an independent dataset. CSI:FingerID will be made available via an easy-to-use web interface. I will also present the new SIRIUS 3 release for computing fragmentation trees using tandem MS data, and results on its performance in identifying the molecular formula of an unknown compound. Finally, I will present preliminary results on compound class identification using tandem MS data. Combining fragmentation trees with molecular fingerprint prediction, we reach previously unmatched identification rates when searching molecular structure databases.

oral 072	15 min	compound ID	Ralf Weber * Early Career Scientist

Optimal multi-stage fragmentation strategies for untargeted metabolite annotation: an experimental and computational assessment

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Multi-stage mass spectrometry (MSn) is often applied to aid annotation and identification of metabolites. The diverse

nature of metabolites and the wide range of experimental parameters involved (e.g. collision energy, depth of fragmentation tree) make the collection of robust multi-stage spectral trees a time consuming and challenging process. Although both collision-induced dissociation (CID) and higher-energy collision dissociation (HCD) applications have been investigated previously for liquid-chromatography and direct-infusion MS, no systematic assessment has been performed to evaluate the optimal set of experimental and processing parameters. Therefore, to maximize annotation performance for untargeted direct infusion MSn fragmentation, a comprehensive assessment is required. Here we use the assignment of elemental compositions (EFs), a critical first step in metabolite annotation, as a benchmark. Automated visual-scripting methodologies were applied to generate instrument method files, each constituting a range of MSn experiments (e.g. CID and/or HCD, normalised collision energy, and fragmentation tree depth). Metabolite standards and LC-separated fractions of human serum were prepared and infused into a hybrid LTQ-Orbitrap Velos (Thermo Scientific) mass spectrometer (nanoelectrospray ionisation (+ve/-ve); Advion). Experimentally collected data dependent high-resolution and low-resolution trees (replicates ≥ 3) were processed based on signal reproducibility. Next, EFs were assigned to each precursor, fragment and neutral loss, and filtered using various constraints and ranked based upon outdegree centrality. Finally, spectral trees were systematically assessed to find the optimal set(s) of parameters to maximise annotation performance for untargeted studies. A spectral library was constructed representing approximately 150 compounds (100–700 Da) of diverse chemistries. Spectral trees covered a wide range of fragmentation parameters, including fragmentation type and collision energy (e.g. top five most intense fragments, CID@10%–60% with steps of five and up to MS5; CID@10%, CID@35% and CID@60% followed by HCD@10%–130% with steps of twenty). Additionally, spectral trees for a cocktail of ca. 15 standards spiked into serum, to mimic a real example, were collected with identical parameters. Accurate discrimination of real signals from noise in MSn trees is one overlooked step in MSn fragmentation workflows. We implemented a number of signal-filtering steps (e.g. retain peaks in r-out-of-x replicates, relative standard deviation threshold for peak intensity and discard electrical noise peaks). Spectral trees processed using this implementation resulted in significantly improved metabolite annotation in comparison to the more common single scan fragmentation tree often filtered with a signal-to-noise threshold. For example, percentage of spectral trees (up to MS3 CID@35%) with a single correct assignment is as follows: ~45% versus ~25% with no improved signal filtering. Some key preliminary findings are: 1) Spectral trees collected in +ve ion mode significantly reduced the number of false positive assignments in comparison to –ve ion mode spectral trees (i.e. using similar annotation parameters, e.g. number of ion types); 2) A minimum of fragmentation level three is required to minimise the number of false assignments for CID; 3) On average certain ranges of CID and/or HCD energies improved annotation; 4) Low resolution spectral trees, even in combination with HR scans, can produce high numbers of false positives; 5) CID and HCD can provide complementary information for annotation. 6) Similar results as above were obtained from serum spiked with standards, which demonstrates the applicability of the presented workflow to real biological samples. Systematic evaluation and optimisation of parameters for untargeted MSn experiments, including a freely-available workflow to process and annotate spectral trees.

oral 073	15 min	compound ID	Robert Mistrik

Metabolomics meets quantum chemistry: a novel strategy towards reliable computer-assisted structure elucidation of metabolites

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The reliable identification of metabolites is imperative, not only in routine metabolite confirmation work but also in untargeted metabolomics' studies. Mass spectrometry based Computer-assisted structure elucidation (CASE) approaches are highly dependent on accurately predicted fragment structures providing the substructural foundation for advanced mathematical and statistical algorithms. Although various combinatorial approaches are widely popular, the prediction of plausible fragment structures reflecting real ion conformations remains a difficult task, limiting the effectiveness of CASE software tools. A novel computational approach based on heuristic and quantum chemical methods has been designed emphasizing the importance of robust prediction of fragmentation processes and ion stabilities. The approach has been continuously employed in a recently launched large-scale fragment assignment project of human metabolic constituents. An important step in establishing the identity of an unknown metabolite is to determine possible substructures derived from predicted ion structures. The ion structure assignment of precursor ions and fragment peaks observed in MSn spectra of human endogenous metabolites is carried out by two sequential processes. First, a list of two dimensional representations of ion dissociation products is generated using both

empirical fragmentation rules and a fragmentation library. Secondly, the relative thermodynamical stability derived from electronic energy is evaluated to distinguish between isomeric fragments having the same molecular formula. The 2D representations in the list are converted to 3D models using well-established methodologies in molecular modeling, such as molecular mechanics (MMFF94), semi-empirical approaches (PM6), and DFT (BLYP,B3LYP). Although for the elucidation of ion structures of complex molecules it is necessary to use site-specific isotope labeling, there are large classes of metabolic compounds of moderate size and structural topology for which accurate mass measurements at the 1 ppm level permit the confident assignment of fragment structures. The presented large scale fragment assignment project aims to annotate thousands of precursor ions and several million fragment peaks present in the mzCloud database, a publicly accessible collection of curated and recalibrated high-resolution, tandem mass spectra of human endogenous metabolites, plant secondary metabolites, food additives, pharmaceuticals and other compounds relevant for metabolomics. The recalibrated peaks typically exhibit mass error equal or close to zero, significantly reducing the possibility of isobaric coincidence of different elemental compositions. To date, a total of 146,602 product ion spectra have been annotated using heuristic methods giving rise to 2,172,212 predicted 2D fragment structures. The absolute stereochemistry of generated fragments is iteratively refined by continuous exploration of the conformational potential energy surface for stationary points. To accomplish this in a cost-effective manner, a series of increasingly demanding structure-optimizing computation stages has been implemented, dynamically reducing the number of low-energy candidates at every stage. Currently, a total of 93,560 and 9,080 3D ion structures have been optimized at the semi-empirical and at the DFT level of theory, respectively. The presented framework offers a major advancement in the prediction of fragment ions observed in mass spectra, in that it can accurately simulate structures in practice emerging in a mass spectrometer by ranking the 2D predicted dissociation pathways based on the potential energy distribution of their 3D equivalent. The data processing pipeline accounts for a high-throughput structural elucidation of hundreds of thousands of plausible product ion candidates using quantum chemical models, a gold standard in ion chemistry. A fundamentally new approach to the comprehensive and reliable metabolite identification critically needed in metabolomics.

oral 074	30 min	diabetes and the metabolic syndrome	Isabel Bondia-Pons

Lipidome as a predictive tool in progression to type 2 diabetes in Finnish men

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Type 2 diabetes (T2D) is a multifactorial metabolic disorder, characterized by hyperglycemia resulting from impaired insulin secretion from pancreatic β-cells and ineffective cellular response to insulin. T2D, with a dramatically prevalence increase, is preceded by a long presymptomatic phase before its diagnosis. Therefore, there is a need for early markers to track progression from a state of normal glucose tolerance through pre-diabetes (impaired fasting glucose and/or impaired glucose tolerance) to T2D. So far, several diabetes risk models and scores have been developed as prognostic tools. However, these are mainly based on established risk factors of T2D and they lack the specificity needed to be used in clinical practice. We applied global lipid profiling at baseline and at 5-year follow-up plasma samples of well phenotyped male participants (107 T2D progressors, 216 matched normoglycemic controls) from the longitudinal study METSIM (METabolic Syndrome In Men) to identify lipidomic profiles of progression to T2D, and to develop and validate a lipid-based predictive model for T2D. Ultra high performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight (QTOF) mass spectrometry (MS) global lipidomic profiling was applied to a total of 1286 plasma samples. Lipidomic profiles from 107 men who developed T2D during the follow-up period and 216 controls were used to build the predictive model, which was validated in a group of 640 men. A total of 280 lipids were identified and included into Bayesian model-based clustering. By applying linear mixed models, eight out of thirteen clusters showed significant differences in their lipid mean levels between cases and controls, after adjusting for BMI. Two opposite patterns were observed. A first pattern with increased mean levels for cases and decreased mean levels for controls, was observed for four clusters consisting only of triglyceride (TG)

species (grouped by different carbon chain lengths and number of double bounds in their structures) and a cluster consisting mainly of phospholipids. The opposite pattern, showed a cluster of sphingomyelins (SM) and a cluster of ether phospholipids (PCe and PEe) with lower mean lipid levels for cases than for controls (adjusted p-value <0.05). Significant lipid clusters were then considered to build a lipid-based predictive model of T2D, by using stepwise logistic regression and random subset cross-validation procedure by assessing classification accuracy calculating the area under the ROC curve for the different models. The performance of a preliminary model, including lipid clusters characterized by medium chain unsaturated TG and SMs, together with several classical T2D risk factors as covariates (age, family history of T2D, waist circumference and SBP) was significantly higher than when compared to the Finnish Diabetes Risk Score (FINDRISC) (AUC=0.81 vs 0.75; p <0.05) The final step will be to select specific lipids that can be representative from the clusters from the previous model, in order to reduce the number of variables of the final predictive model. The analysis of the plasma lipidome improves the predictive accuracy of T2D models which are based on established risk factors

oral 075	15 min	diabetes and the metabolic syndrome	Weidong Zhang * Early Career Scientist

Metabolomics analysis reveals that arginine is depleted in knee osteoarthritis patients

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Osteoarthritis (OA) is the most common form of arthritis and results in substantial morbidity and disability in the elderly and imposes a great economic burden on society. Currently, there are no generally accepted disease-modifying interventions capable of slowing or reversing the disease progression, partly because there is no reliable method to detect early changes of the disease. Therefore, there is a pressing need to develop reliable biomarkers that can inform on the process of joint destruction in OA. The aim of the study was to identify novel biomarkers for OA using a metabolomics approach. We utilized a two-stage case-control study design. 64 knee OA patients and 45 controls were included in the discovery stage and 72 knee OA patients and 76 age and sex matched controls were included in the validation stage. Plasma samples were collected after overnight fasting. Targeted metabolite profiling was performed using Biocrates AbsoluteIDQ p180 kit coupled with UPLC-MS. Linear regression was used to identify novel metabolic markers for OA. ROC analysis was used to examine diagnostic value of the novel OA marker(s). Gene expression using real-time quantitative PCR was performed on human cartilage tissues obtained from 32 OA patients and 21 healthy controls to explore the potential mechanism for the novel OA marker(s). We found that 18 metabolites were significantly associated with knee OA with p≤2.9×10-4 in discovery stage after adjustment of sex, age and BMI. Six of them were confirmed in our validation stage. Those six metabolites were arginine, lysoPC a C28:1, PC aa C36:6, PC ae C36:2, PC ae C38:0, SM (OH) C14:1. Arginine was the most significant metabolite in both discovery and replication stages with p = 3.5×10-13 and 5.6×10-33, respectively. Knee OA patients had on average 69 μM lower of plasma arginine concentration than controls after adjustment for age, sex, and BMI. ROC analysis showed that arginine had the greatest diagnostic value with AUC of 0.984. The optimal cutoff of arginine concentration was 57 μM with 98.3% sensitivity and 89% specificity. The depletion of arginine in OA patients were most likely due to the over activity of arginine to ornithine pathway as we found both ornithine and proline concentration were higher in OA patients than in controls (158 μM vs 72 μM, p=1.1×10-22 and 231 μM vs 193 μM, p=4.6×10-4). Proline is precursor to sustain collagen synthesis in humans, thus depletion of arginine in OA patients reflects the body's effort to repair the degraded cartilage. Furthermore, arginine is a nature inhibitor of cathepsin, a protease that breakdown cartilage. We found that expression of cathepsin K and B were 636% and 36% increased in OA-affected cartilage than that in controls (all p<0.015), respectively, which was most likely due to the over activated TGF-β signalling as both cathepsin K and B were highly correlated with expression of TGF-β1 in OA-affected

cartilage (rho=0.62 and 0.48, respectively, all p<0.005). Thus, the depletion of arginine in OA patients reduced its inhibitory effect on cathepsin and led to cartilage breakdown. Our findings indicate that arginine have a great potential to serve as diagnostic marker and in treating knee OA patients.

oral 076	15 min	diabetes and the metabolic syndrome	Annalaura Mastrangelo * PhD student

Bariatric surgery outcome predictors: evaluation of metabolic changes and food craving by a non-targeted metabolomics approach.

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Bariatric surgery (BS) is the most effective and durable treatment for obesity and its associated comorbidities. Although the underlying mechanisms of its remarkable efficacy are still unclear, it has been highlighted that the anatomical restriction imposed by the surgery might not be the unique effector, but rather a contributing factor in conjunction with metabolic alterations taken place in the body at different levels (i.e. food absorption, microbiota and brain activity). In the present study we have applied a multiplatform metabolomics approach to gain a better understanding of the extent to which other factors are associated with bariatric surgery efficacy in order to identify potential outcome predictors, thereby uncovering new targets for less-invasive treatments. Metabolomics analysis was performed in serum samples from healthy volunteers (group 1 n=23, BMI < 25), obese patients before surgery (group 2 n=23, BMI ≥ 40) and one year after surgery (group 3 n=23, average BMI=31 kg/m2) by UHPLC-ESI-QTOF-MS, GC-EI-MS-Q and CE-ESI-TOF-MS (Agilent, USA). In addition, food craving, following the Food Cravings Questionnaire trait scale (FCQ-T), was evaluated for groups 1 and 2. To estimate the strength of the linear dependence between the observed metabolites and food-craving, linear correlation was measured by the Pearson correlation coefficient. Finally, group 2 was divided in two subgroups according to high (score ≥ 15, n=13) and low food craving (score < 15, n=10) to predict a possible discrimination among subjects after surgery. The multiplatform approach including complementary high resolution techniques, allowed the detection of 190,330 potential compounds. Once mined the valuable information from the data matrix, chemometric tools (correlation, univariate and multivariate statistical data analyses) were applied to investigate the specific metabotype of the groups. We have explored the metabolic changes associated with the gastric bypass surgery (2vs.3) and with the recovery after surgery (3vs.1). 84 compounds (2vs.3) and 41 compounds (3vs.1) showed statistically significant differences (i.e. p <0.05 after FDR) between groups. Among these metabolites, bile acids and gut microbiota by-products (secondary bile acids, p-cresol, benzoate, indolelactate) presented the most remarkable changes, reflecting the great influence of the microbial community on the surgery outcome. Moreover, we observed that after surgery, the lipid profile (fatty acids, monoacylglycerides and phospholipids) was modified with a recovery toward normal condition, supporting BS efficacy in promoting metabolic improvements. Regarding the recovery evaluation model (3vs.1), we noted differences in the metabolic profiles pointing out the necessity to explore other factors that might influence the outcome of the bariatric procedure. Therefore, we have correlated the psychological factor, food-craving, with the metabolic profile of normal weight and obese subjects (groups 1 and 2). Among all metabolites that were highly correlated between these parameters, we have selected those specific of each group -20 (group 1) and 19 (group 2)- as they might reflect a different brain behavior associated to a specific metabotype. Finally, we have investigated the food-craving predictive capability for the discrimination of the individuals after surgery. Using the Shared and Unique (SUS) plot (SIMCAP+), we have highlighted potential metabolites associated with food-craving (i.e. carnitines, fatty acids, aminomalonate and phospholipids), surgery (phospholipids, amino acids and organic acids), and those common between the two models (p-cresol, taurodeoxycholate, serine, glycerol). Metabolomics points to microbiota metabolites and food-craving evaluation as potential contributor factors to predict the surgical outcome of BS.

oral 077	15 min	diabetes and the metabolic syndrome	Kelli Sas * Early Career Scientist

Altered metabolic nutrient utilization and mitochondrial dysfunction characterizes diabetic nephropathy

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Diabetes is associated with microvascular complications such as diabetic nephropathy (DN). DN is the most common cause of end-stage renal disease in the United States. Diabetes results in increased glucose and free fatty acid levels in the kidney, and while it is well-recognized that diabetes leads to altered carbohydrate, amino acid and fatty acid metabolism, the utilization and flux of these substrates in vivo has not been systematically studied. Understanding the metabolic abnormalities of DN will provide a better understanding of the mechanisms resulting in DN and may highlight therapeutic targets for treatment or prevention. To obtain a global systems view of fatty acid metabolism in the kidney, we utilized gene expression studies in parallel with targeted metabolomics approaches in the BKS db/db animal model of DN. We developed a comprehensive targeted metabolomic platform for simultaneous quantitative analysis of glycolytic, tricarboxylic acid (TCA) and fatty acid oxidation intermediates by liquid chromatography tandem mass spectrometry (LC/MS/MS). In vivo metabolic flux analysis was performed following gavage of 0.5 g/kg U13C palmitate to study the dynamic changes in fatty acid metabolism. Seahorse extracellular flux analysis of mitochondria isolated from renal cortex was used to assess mitochondrial function. Transcriptomics identified significant upregulation of pathways involved in β-oxidation and fatty acid metabolism. Consistent with the transcriptomics data, steady state levels of acylcarnitines were significantly increased in diabetic renal cortex and mitochondria from renal cortex (1.5 to 6-fold). Metabolic flux analysis showed a significant elevation of palmitate uptake and labeled long-chain acylcarnitines and TCA cycle intermediates in the diabetic kidney, consistent with increased fatty acid flux. Acetylation of enoyl-CoA-hydratase/3-hydroxyacyl-CoA dehydrogenase (EHHADH) in the diabetic renal cortex was increased, showing activation of a primary enzyme in β-oxidation. Mitochondria from diabetic renal cortex exhibited reduced ATP production, impaired complex 2 activity and increased proton leak indicating diminished activity. Taken together, these results show a metabolic reprogramming in the diabetic kidney leading to mitochondrial dysfunction and oxidative stress. We identify a role for altered fatty acid metabolism in the pathogenesis of DN and suggest a novel therapeutic target.

oral 078	15 min	diabetes and the metabolic syndrome	Kallyandra Padilha * PhD Student

SERUM METABOLOMIC PROFILE OF DIABETIC BRAZILIAN INDIVIDUALS

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The development of common forms of diabetes comes from the interaction between environmental and genetic factors and it is well known that related complications showed by these patients are always associated with underlying metabolic perturbations, even before the blood glucose level had progressed far enough to be diagnosed. Metabolomics profiles have been recently used in different studies to identify metabolic signatures of T2DM, metabolites associated with disease progression and response to treatment. In this population-based metabolomics study, we aimed to analyze metabolites from T2DM and healthy subjects from the general Brazilian population, using

GC/MS. In addition, we identified different serum metabolic patterns between groups and association of these metabolites with molecular pathways changed by the disease. 893 subjects participants of “the Baependi Hearts Study” participated in this study. Subjects were split into two groups: diabetic (91 individuals, glucose >126mg/dL) and nondiabetic (802 individuals). Serum samples were deproteinized with methanol, followed by lyophilization and derivatization. The extract was spiked with 50uL methoxyamine in pyridine solution. This methoximation reaction was performed at RT, followed by trimethylsilylation adding MSTFA/TMCS. For each samples was add 5µL of internal standard solution (d27-myristic acid). 1µL of this derivative was used for GC/MS analysis by GC-MS (Agilent 5977A Series GC/MSD). Analysis was performed with FiehnA.01.00 and NIST 11 compound library using Unknowns - Agilent MassHunter Workstation Quantitative Analysis. Data were normalized using LOESS method according to injection order and analysis day. Our sample showed the same pattern of diabetes prevalence of the total Brazilian population. By GC-MS technique, normalization and statistical analysis it was possible to identify differentially changed metabolites in serum of diabetics and non-diabetics. Metabolite profiling identified 156 metabolites upregulated in T2DM subjects compared to heathy individuals. According to system biology analysis, these changed metabolites are related to Amino acid metabolism, Metabolic disease, Obesity, Vitamin and cofactor metabolism, Protein degradation, Energy metabolism and Calcium regulation. Our data suggest that these metabolic pathways are altered in diabetic subjects. Some metabolites identified such as BCAA are well described in the diabetic´s literature and are related to insulin resistance. Analyzing just the patients from T2DM group, it was possible to separate patients in use of the antidiabetic drug (Metformin) from those not using the medication according to their metabolite profile. We identified maltose, tyrosine, alanine, glutamine, 2- piperine carboxylic acid, eicosenoic acid, asparagine as upregulated in treated subjects; and ornithine, glycine, hipotaurine, tryptophan, cadaverine, tocopherol, tetradecanoic acid and butanedioic acid uregulated in non-metformin user diabetic individuals. The metabolic profile from healthy subjects was closer to the one of metformin treated patients. Aiming to identify metabolites related to early T2DM, we compared metabolic profile between healthy subjects with high fasting glucose level (between 100 and 125mg/dL) and healrhy individuals with normal glucose levels (less than 100mg/dL). 150 metabolites were different between these groups. These metabolites could be useful as biomarkers in early T2DM diagnosis and monitoring of treatment. Serum metabolomic profiling in a large Brazilian population-based study.

oral 079	30 min	plant metabolism	Sunitha Shiva * Early Career Scientist

Identification of genes and enzymes responsible for the stress-induced modification of membrane lipids in Arabidopsis thaliana

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Apart from maintaining cellular integrity and separating intracellular compartments, membrane lipids are dynamically and extensively modified during plant growth and exposure to environmental cues. Our research goal is to identify genes/enzymes involved in inducing lipid modifications, particularly under stress. The approach involves screening of candidate knockout mutants with wounding as the applied stress. 384 Arabidopsis thaliana knock-out mutants and wild type plants were grown in growth chambers under a 14/10 h light/dark cycle at 21°C with 60% humidity. All were planted in triplicate. At 30 days of age, an unwounded leaf was harvested from each plant and immediately two other leaves were subjected to mechanical wounding with a hemostat. One of the wounded leaves was harvested 45 min later. After a one-step extraction, membrane lipids and their derivatives were analyzed with direct-infusion mass spectrometry with multiple reaction monitoring (MRM). Quality control (QC) samples, analyzed throughout, were used for data normalization. Data were acquired on a Xevo-TQS “triple quadrupole” mass spectrometer. Wild type Arabidopsis thaliana subjected to wounding stress produce acylated galactolipids (acGLs), acylated

phosphatidylglycerol (AcPG), acylated steryl glucosides (ASG), acylated phosphatidylethanolamine (NAPE), lipids with oxidized fatty acyl chains, head-group acylated membrane lipids, monoacyl polar lipids, phosphatidic acids, and triacylglycerols (Vu et al., 2014, Plant J., 80, 728-743 and unpublished). Initial screening of 68 KO mutants has identified 8 KOs with lipid profiles significantly different from WT; compositions of four are more different from WT in wounded leaves than in unwounded leaves. The approach and interesting identified KOs will be discussed. Candidate genes involved in stress-induced lipid modification were identified by analyzing a broad range of lipid modifications by mass spectrometry.

oral 080	15 min	plant metabolism	Wolfram Weckwerth

Ecological Metabolomics – from model systems in the lab to natural populations in the field

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The experimental high-throughput analysis of molecular networks enables the comprehensive characterization of acclimation strategies of plants in a changing environment. However, recent studies have demonstrated that it is hardly possible to predict an in situ metabolic or phenotypic output from highly controlled phytotron or greenhouse experiments. To overcome this limitation, we present an integrated metabolomics approach for the comprehensive analysis of primary and secondary metabolite compositions of in situ samples of natural Arabidopsis thaliana populations. Characteristic metabolic signatures could be identified from secondary metabolite profiles as well as form the covariance information of primary metabolite levels. In summary, the presented integrative metabolomics approach enables the differentiation between Arabidopsis in situ populations being crucial for deriving a valid genotype-phenotype relation. In situ leaf samples of Austrian Arabidopsis thaliana populations were collected in the field and immediately snap frozen in liquid N2. Central polar primary metabolites, such as sugars, carboxylic acids and amino acids, were analysed by GC-MS while secondary metabolites were detected in a LC-MS/MS approach according to Doerfler et al., 2013. SNP-based genotyping was performed using 100bp paired-end reads on an Illumina HiSeq platform. The functional integration of GC-MS metabolomics data into a metabolic network was performed, as previously described (Doerfler et al., 2013; Nägele et al., 2014), by the approximation of the biochemical Jacobian matrix. This approximation directly connected the experimental covariance information with a metabolic network structure derived from Arabidopsis genome information. Genotyping based on single nucleotide polymorphisms (SNPs) confirmed a natural genetic distance within the sampled in situ populations. Further, comparing genomic data to a global set of Arabidopsis accessions (http://1001genomes.org/), accessions from Italy and Czech Republic were found to be most similar. Sparse partial least squares (sPLS) regression analysis of 41 absolutely quantified primary metabolites indicated only a weak separation of nearby grown in situ populations. This separation was found to be mainly due to differential levels of organic acids. To investigate not only the metabolic homeostasis, but also its biochemical regulation, the metabolic covariance information was linked to the biochemical network structure finally resulting in entries of biochemical Jacobian matrices. Median values of entries of these matrices were subjected to principal component analyses (PCA), showing a clear separation of the natural populations. PCA loadings related to the biosynthesis of aromatic amino acids indicated potential differences in the regulation of secondary metabolism. Analysis of relative secondary metabolite abundances confirmed the indicated prediction showing a clear separation of the populations. Several of the secondary m/z-features being responsible for the clustering of the samples could putatively be annotated to anthocyanins attached to sinapoyl moieties. In summary, our findings provide evidence for the suitability of an integrated GC/LC-MS/MS approach for the characteristic differentiation of in situ samples of natural Arabidopsis populations. A differentiation solely based on levels of primary metabolites was not successful, indicating the need for a combination with information of secondary metabolism and biomathematical methods for comprehensive data integration. Finally, we provide a metabolomics platform which provides characteristic insights into molecular phenotypes of natural populations of Arabidopsis

thaliana which promotes our understanding of the genotype-phenotype relationship in plants. Literatur: Doerfler et al. (2013) Metabolomics 9: 564-574 Nägele et al. (2014) PLoS One 9: e92299 A comprehensive metabolomics approach enables the characteristic differentiation and in situ molecular phenotyping of natural Arabidopsis populations.

oral 081	15 min	plant metabolism	Lei Wang * PhD Student

Integration of metabolomics and proteomics to understand the molecular physiology of cacao beans during ripening

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The seeds of Theobroma cacao are the original material for chocolate production and possess enormous market value. Several studies have focused on chemical content of cacao beans of different varieties (Elwers et al., 2009) or the beans after processing procedures (Counet et al., 2004; Misnawi and Ariya, 2011) for industrial and economic perspective. In contrast, only few studies focused on elucidating the dynamic developmental processes (Bucheli et al., 2001; Pereira-Caro et al., 2013). Seed development is a highly dynamic process with complex physiological and biochemical interactions. The overview of metabolome and proteome profiles during cacao seed development facilitates the comprehensive and systemic understanding of cacao seed ripening process. Metabolites and proteins were extracted from the developing cacao seeds based on a universal isolation protocol (Weckwerth et al., 2004) with some modifications. The polar phase of metabolite extract was measured with both GC-TOF-MS and LC-Orbitrap-MS/MS platforms to obtain the primary and secondary metabolism profiles. Metabolite identification and relative quantification were performed with the software LECO Chroma TOF, Xcalibur and LCquan. Protein was digested with trypsine. LC-Orbitrap-MS/MS platform was applied for the peptide detection. Proteome Discoverer (v 1.3, Thermo Scientific) was employed to search a raw data against a cacao fasta file including 44 404 proteins. COVAIN was used for the integrative and multivariate statistical data analysis (Sun and Weckwerth, 2012). Metabolomic and proteomic analysis of cacao beans from four developmental stages allowed, in total, identification and relative quantification of 127 metabolites and 472 protein candidates. Multivariate statistical analysis of the comprehensive metabolome (including sugars, amino acids, organic acids, alkaloids, flavonoids, and N-phenylpropenoyl amino acids) and proteome data set revealed distinct dynamic patterns of cacao beans during development. Metabolism switches unveiled the biochemical adaptation of cacao seed during development. In the early development stages, protein candidates related with cell division were in high abundance which indicated the seed growth is mainly based on fast cell division at this phase. On the metabolome level, sucrose that transported from leaves primarily hydrolyzed to fructose and glucose to fulfill the enormous demand of energy. Jacobian matrices which were calculated from mathematical model including pathway information (Doerfler et al., 2013; Nägele et al., 2014) revealed significant induction of aromatic amino acid metabolism and a reprogramming of TCA cycle from stage 1 to stage 2. Either principal component analysis or stage-specific comparison of co-variance derived Jacobian entries revealed that stage 2 is an outstanding and crucial phase for the cacao seed to accumulate sufficient energy and precursors for the later synthesis of secondary metabolites and storage molecules. During maturing, purine alkaloids, flavonoids and raffinose accumulation endowed cacao seed a stringent taste and provided cacao seed stronger resistance to abiotic stresses. A significant reprogramming of sugars and the essentiality of aromatic amino acids accumulation prior to the induction of flavonoids biosynthesis were observed during cacao development. The unveiling of interactions between primary metabolism and secondary metabolism, metabolite and proteins during cacao seed development by integrative analysis of proteome and metabolome data presented us a comprehensive dynamic development process during cacao seed ripening. Cacao seed development described with dynamic metabolomic and proteomic profiles revealed a significant shift in flavonoid biosynthesis during early stages.

oral 082	15 min	plant metabolism	Georg Jander

Metabolomic and transcriptomic responses of maize to aphid feeding

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More than 90 herbivorous insect species are known to attack maize (Zea mays), resulting in losses ranging from 6% to 19% of total productivity. Aphids and other hemipteran pests cause direct damage to maize by sucking phloem nutrients as well as indirect damage by transmission of plant viruses. To survive insect attack, maize plants have evolved constitutive and inducible chemical and physical defenses. We aimed to elucidate the metabolic processes that are induced in maize leaves during corn leaf aphid (Rhopalosiphum maidis) attack, and thereby identify previously unknown aspects of maize defense against insect herbivory. Leaves of maize inbred line B73 were infested with corn leaf aphids and leaf samples were collected at time points ranging from 0 to 96 hours. Gene expression profiling by Illumina sequencing (RNAseq), HPLC, HPLC-MS, and GC-MS were used to generate large transcriptomic and metabolomic data sets. Metabolites, genes, and metabolic pathways that were strongly up-regulated or down-regulated in response to aphid feeding were identified. Expression of specific up-regulated maize genes was knocked out with Ds transposon insertions. The effects of these mutations were analyzed using measurements of changes in metabolite abundance and insect bioassays. Analysis of metabolite and gene expression data showed that maize responses to aphid feeding are a two-stage process. In the first few hours after aphid feeding, there is a strong induction of defense responses and a reduction in primary metabolism, including photosynthesis and amino acid biosynthesis. After about 24 hours there is a further metabolic switch and many defense-related metabolites and gene expression levels revert to basal levels. For instance, jasmonic acid, a plant defense signaling molecule is signaling molecule is significantly elevated after 4 hours of aphid feeding, but then reverts to pre-feeding levels. This observation suggests that aphids may be able to suppress plant defense responses after a period of feeding. Production of benzoxazinoids, a class of defense-related metabolites in maize was increased in response to aphid infestation. Knockout mutations in the benzoxazinoid biosynthesis pathway promoted aphid reproduction. A knockout mutation of an aphid-induced terpene synthase shifted the terpene profile of the maize plants and had a negative effect on aphid reproduction. This suggested that the wildtype terpene profile of maize inbred line B73 is more attractive for aphids and/or that the mutant maize terpene profile contains compounds that are toxic or deterrent. Previously unknown genes and metabolites contributing to maize aphid resistance were identified.

oral 083	15 min	plant metabolism	Kris Morreel

Glycosylated lignin oligomers are stored in Arabidopsis leaf vacuoles.

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Lignin is an aromatic polymer that is present in the cell walls of secondarily-thickened cells such as vessels and fibers, providing growth-supporting and water-conducting properties to these cells. Lignin is formed via combinatorial radical-radical cross-coupling reactions of the monomers, i.e. the monolignols. A variety of coupling reactions are known to occur, yielding different units in the final polymer. The seemingly randomness of these reactions as well as the necessary oxidative conditions and the absence of any contradictory evidence up to now, has lead to the general idea that these radical reactions are restricted to the apoplast. However, the observation of glycosylated lignin oligomers in recent studies has doubted this assumption. Vacuoles of Arabidopsis, prepared using a Ficoll gradient, were extracted with methanol and profiled via reversed phase UHPLC-FT-MS. In addition, co-feeding experiment of leaf protoplast cultures were performed using 13C-labeled coniferyl alcohol and 13C-labeled dimers of coniferyl

alcohol. Intracellular coupling of monolignols was also explored via comparative profiling of wild types and Arabidopsis mutant down-regulated for either caffeic acid O-methyltransferase, caffeoyl-CoA O-methyltransferase or cinnamic acid 4-hydroxylase, and of wild type poplar and poplar down-regulated for phenylcoumaran benzylic ether reductase. Based on liquid chromatography-Fourier transform mass spectrometry (LC-FTMS) profiling of extracts from purified Arabidopsis leaf vacuoles, oligolignol glycosides were found to be stored in the vacuole. This prompted the idea that the synthesis of “lignin” oligomers might as well occur intracellularly. Support for this hypothesis was obtained via protoplast co-feeding studies using stable isotopes of mono- and dilignols. Complementary, in vivo evidence for the occurrence of intracellular monolignol coupling in plants was also obtained from the accumulation of cysteine-bearing dilignols in phenolic profiles of transgenic poplars down-regulated for phenylcoumaran benzylic ether reductase (PCBER) as compared to wild type poplar. The cysteine-bearing dilignols could only be reasoned to arise from intracellular coupling as a consequence of the presumably enhanced oxidative environment in these transgenic poplars as compared to wild type poplars. a previously unknown pathway is uncovered enabling plants to create an arsenal of natural compounds in a very efficient way.

oral 084	30 min	healthy aging	Hannelore Daniel

Food meets human metabolism

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Throughout an average human lifespan about 30 tons of food are consumed and pass the human metabolic system. Foods (and drinks) can be simple comprising only a few ingredients but can reach highest complexity in composition. When assessing the interplay of food with the human system on background of its genetic make-up, the most crucial aspect is the lack of proper methods to identify and quantify the various food items consumed. Food intake assessment is usually done via a food frequently questionnaire that requests the documentation of food items and amount eaten with nutrient intake calculated based on food composition data bases. Metabolomics approaches with objective measures of marker compounds that reflect intake of distinct food items (and allow quantification) by analysis of blood, urine or breath (volatiles) are promising as new assessment tools. In addition to these markers of exposure, endogenous metabolites produced from nutrients and nutrient conversion processes may serve as biomarkers with predictive quality in the health-disease trajectory There are in essence 3 categories of organic food constituents that are accessible by metabolomics approaches in whatever type of human biosample. Macronutrients (carbohydrates, lipids) which when oxidized are no more detectable whereas amino acid oxidation leads to urea/nitrogen and sulfuric acid (from methionine and cysteine) as products. The second category are nutrients such as the vitamins which are excreted in feces and urine either un-modified or after metabolic conversion (oxidation, conjugation) including transformation by gut microbiota. The third category comprises the most characteristic compounds. They are per se not nutrients. This category comprises for example in plant-based food all intermediates of secondary metabolism with > 10.000 unique structures. Those compounds usually undergo substantial modification either in human xenobiotic phase I or phase II metabolism or via the microbiome and/or combinations of both. In food of animal origin the number of characteristic compounds is in contrast very limited. I shall be using examples from all categories to demonstrate the benefits but also the limits of current metabolomics approaches on the interface of food and human metabolism. Food, new exposure markers, new biomarkers

oral 085	15 min	healthy aging	Jonas Zierer * PhD student

Metabolomics profiling reveals novel marker for leukocyte telomere length

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Telomeres are nucleoprotein structures capping the chromosomes and protecting their end from deterioration during somatic cell replication. Leukocyte telomere length (LTL) is known to decrease with age at a rate of 20–40 base pairs

per year and it is considered one of the most accurate predictors of biological ageing. Indeed, LTL has been implicated in a spectrum of ageing related diseases such as hypertension, myocardial infarction, congestive heart failure, vascular dementia, osteoporosis, osteoarthritis and Alzheimer's disease independently of age. Genome-wide association studies have successfully identified a number of genes associated with LTL. Here, we aim to further investigate the pathways regulating LTL using a metabolomics approach in a large cohort of individuals from the UK. To this end, we performed non-targeted mass spectrometry based metabolomic profiling of blood samples from 3190 individuals in the TwinsUK cohort for which measures of LTL were available through qPCR. We studied the correlation between the levels of 280 identified metabolites and LTL in the larger population using linear mixed models adjusting for age, sex, BMI, family relatedness and multiple testing using Bonferroni correction (P<2e-4). We then validated our top results in an independent group of 57 monozygotic pairs of twins who were discordant for LTL. Results from both analyses were meta-analyzed using inverse variance fixed effect meta-analysis. Finally, the significant metabolites were tested for association with ageing-related phenotypes such as HDL cholesterol and lung function after adjustment for age. Two dipeptides (gamma-glutamyltyrosine: meta-analysis beta [95%CI]= -0.01 [-0.02:-0.01], P=2.55e-6 and gamma-glutamylphenylalanine: beta=-0.01 [-0.01:-0.00], P=4.90e-5), one lipid (1-stearoylglycerophosphoinositol: beta=-0.02 [-0.03:-0.01], P=1.03e-6) and one xenobiotic (4-vinylphenol sulfate: beta=-0.03 [-0.04:-0.01], P=9.27e-5) were significantly associated with LTL after adjustment for age and multiple testing and were replicated in the independent group of twins. The two dipeptides are strongly associated with renal function (gamma-glutamyltyrosine: beta=-1.64 [-2.04:-1.24], P=1.6e-15 and gamma-glutamylphenylalanine: beta=-2.31 [-2.69:-1.92], P= 1.04e-31), lung function (beta=-0.02 [-0.04:-0.01], P=5.50e-4 and beta=-0.04 [-0.05:-0.03], P=3.23e-10 respectively), HDL cholesterol (gamma-glutamylphenylalanine: beta=-0.03 [-0.04:-0.02], P=4.53e-8) and blood pressure (gamma-glutamyltyrosine: beta=0.40 [0.12:0.69], P=4.69e-3). Gamma-glutamylamino acids are produced by gamma-glutamyl transpeptidase (GGT), which transfers the gamma-glutamyl residue from glutathione (GSH) to the amino acid. Higher levels of gamma-glutamyl amino acids, which were found to be associated with shortened telomeres, could be a sign for increased GGT activity. This has been observed in several tumors and is associated with increased oxidative stress, probably due to alterations of the GSH metabolism. Accordingly, gamma-glutamyltyrosine is a putative biomarker of oxidative damage to proteins. The lipid 1-stearoylglycerophosphoinositol belongs to the group of glycerophosphoinositols, which are bioactive lipids and have been implicated in the activation of the Ras signalling pathway. Thus, they also might play a role in oxidative stress, cell proliferation and tumor development. Moreover, in our cohort 1-stearoylglycerophosphoinositol is associated with blood pressure (beta=0.59 [0.34:0.84], P=2.86e-6) and lung function (beta=-0.02 [-0.03:-0.01], P=1.72e-4). 4-vinylphenol is a xenobiotic that has previously been found to associate with tobacco smoking and might thus provide a link between reduced LTL, decreased lung function and smoking. Our results indicate an involvement of increased oxidative stress in human biological ageing, reflected by shortened telomeres and ageing phenotypes. However, whether the observed changes in the blood metabolome are causes or consequences of telomere shortening remains subject to future studies. The novel associations identified between blood metabolites and LTL may provide targets to understand the molecular mechanisms involved in ageing.

oral 086	15 min	healthy aging	Nicholas Rattray * Early Career Scientist

The fRaill Project – Investigating Ageing and Frailty in Later Life using Untargeted Serum Metabolomics

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The impact of ageing well in later life is rapidly developing into a key strategy of public health policy. Well-being and frailty are theorised to be interlinked to natural and socioeconomic inequalities; thus addressing this area is an important step towards meeting the challenges posed by the worlds ageing populations. The fRaill Project (http://www.micra.manchester.ac.uk/research/fraill/) is a multilayer analysis of socioeconomic/biological determinants to develop an integrated understanding of processes leading to positive and negative outcomes in later life. Empirical

data and clinical samples from the English Longitudinal Study of Ageing (ELSA) have been used to examine genetic influences and their relationship with markers of metabolic processes to aid in the identification of biological characteristics that relate to the concept of frailty. Within the ELSA resource are serum contributions from over 6000 participants at two different time points. Following metadata screening, samples from 1200 subjects were identified as targets for metabolomic analysis as they contain full cross sectional data-sets from social, genetic and physical studies. These studies have determined where each contributor sits on the Rockwood frailty index (a clinical assessment of frailty) with serum samples having been analysed on GC-ToF-MS (Leco PEG 3) and UHPLC-MS (Orbitrap-XL) metabolomic pipelines. A subsequent deterministic approach to statistical analysis using a combination of univariate and multivariate tools (PC-DFA, Lasso/PLS-Regression/TW-ANOVA) and has been employed to develop a series of metabolic frailty phenotypes. At the time of writing we have carried out analysis on the first wave with 1200 serum samples having been analysed on both MS systems. With the combination of statistical tools supplying a cross-sectional view on how metabolic profiles shift from healthy to frailty phenotype, data from subsequent network analysis allows enriched networks to be used as a tool to allow metabolomics to inform the development of a targeted genetic approach on already performed GWAS data. If this system is proven to be successful, this metabolomics approach will then be applied longitudinally on a composite set of 1200 samples taken at a time point 4 years later. The end goal is to see if we can track and predict the progression of frailty over time within the ELSA cohort. If a strong link is present then we propose it may be possible to predict a specific genetic, hormone and metabolic phenotype to frailty and potentially use metabolic/genetic screening to help predict those most at risk in the future and design informative interventions. This study is the first large scale metabolomics analysis in ageing research to investigate the concept of frailty in humans.

oral 087	15 min	healthy ageing	Julijana Ivanisevic * Early Career Scientist

Metabolomics Implicates Warburg Metabolism in the Aging Brain

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Brain structure and function are rigorously dependent upon adequate energy supply and regulation of signal transduction pathways. However, it is still unknown how the energy demand and signaling pathways necessary for healthy brain function are affected during the normal aging process in mammals. A systems biology characterization of metabolite homeostasis throughout the aging process would provide important insight into brain metabolism and potential impairment related to energy metabolism dysfunction as a hallmark of aging. We have applied the cutting-edge, mass spectrometry-based ‘omic technologies to reveal metabolic changes that are taking place across an aging mouse brain. Focused beam microwave irradiation (FBMI) was applied to deliver a high power pulse to induce instant euthanasia, simultaneously halting brain enzymes. Effectiveness of FBMI was validated in each animal using noninvasive postmortem 1H-MRS of brain tissue. A complex brain metabolome was successfully recovered by HILIC-ESI-MS on Q-TOF iFunnel 6550 mass spectrometer. The proteome data were acquired on a TTOF 5600 mass spectrometer and the values were derived from a super-SILAC experiment where heavy labeled cell mitochondria are added to experimental non-labeled samples as an internal standard allowing for quantification. The integration of metabolome data and proteome expression data from different anatomical regions of brain enabled us to define a temporal brain metabostasis and aging progression by characterizing the energy metabolism and signaling pathways that are implicated in the normal aging process of the brain. Over 50 discriminating (significantly altered) metabolites that represent the readout of the aging process in the brain were identified (MS/MS matching), pathway mapped and correlated with the quantified proteins. A predicted metabolic activity network in healthy aging brain was defined by 4 subnetworks or modules represented by glutamate, AMP, NAD and GDP-GTP that showed more internal connections than expected randomly in the whole network. Cellular energy homeostasis in brain was found to be compromised as reflected in significant accumulation of purines, pyrimidines, nucleosides and nucleoside monophosphates on one hand and depletion of nucleoside di- and tri-phosphate levels on the other. Network analysis, pathway mapping and directional identification of individual changes highlighted the altered nucleotide biosynthesis and degradation, and energy metabolism, including glycolysis, TCA cycle and oxidative phosphorylation,

with parallels to Warburg reprogramming. A few signaling pathways were also affected, including adenosine receptor signaling that combined with the changes in energy currency metabolites and amino acids implies the dysregulation of dopamine metabolism. Global profiling of brain metabolome across time and different anatomical regions provided an insight into normal aging process in brain.

oral 088	15 min	healthy ageing	Bruce Watkins

Interplay of nutrition and metabolomics to identify characteristic metabolite features that support healthy aging

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Metabolomics is a robust means of establishing links between nutrition and health status. In older adults, sarcopenia, with declines in muscle mass/function, are risk factor for frailty, disability, and mortality. Underlying causes are not clear but include muscle atrophy, inflammation, and oxidative stress. Increasing muscle fat content and inadequate nutrition contribute to muscle wasting. Polyunsaturated fatty acids (PUFAs) are nutrients that have a direct effect on energy metabolism and levels of bioactive lipids. The PUFAs can affect flux through metabolic pathways, participate in inflammatory processes, and serve as endogenous ligands to activate cannabinoid receptors to induce eating and fat deposition. The complex biological relationship of PUFAs and aging was examined using metabolomics in cell cultures, rodents, and the human. In cell culture, mouse, and human experiments endocannabinoids and oxylipins were measured in samples, filtered, and analyzed by ultrahigh performance liquid - electrospray ionization tandem mass spectrometry (UPLC-MS/MS) by back-to-back (+)-/(-)-mode injections. Non-targeted global metabolite profiling was performed using three independent platforms: UPLC-MS/MS) for: 1) basic species; 2) acidic species; 3) gas chromatography/MS. Tissue fatty acid composition was measured by GC of fatty acid methyl esters. mRNA for gene expression in cell cultures and mouse tissues were determined by quantitative real-time polymerase chain reaction. All data were analyzed for significance in SAS for Windows version 9.3, SAS Institute Inc., (Cary, NC) followed by appropriate mean difference tests ($P < 0.05$). Omega-3 PUFAs may support lean muscle mass and direct metabolism that minimizes fat accumulation and insulin resistance. Metabolomics and gene expression were used to examine the effects of PUFA on the endocannabinoid system (ECS) and muscle mass and fat accretion. Primary and differentiated C2C12 myoblasts were treated with docosahexaenoic acid (DHA), docosahexaenoylethanolamide (DHEA), arachidonic acid (AA), arachidonoylethanolamide (AEA), CB1 antagonist NESS0327, or vehicle control (VC). C2C12 cultures exposed to DHA and DHEA had higher glucose uptake and differential ECS gene expression compared to AA-derived ligands. Human primary myoblasts treated with cannabinoid agonist AEA and DHEA as well as NESS0327 showed increased intracellular glucose and lactate. C57BL/6 mice fed a semi-purified diet containing DHA had lower epididymal fat mass, but equivalent body weights compared to controls. Global metabolite (GM) profiles indicated that DHA feeding induced a fuel switch from glucose toward fatty acid oxidation. Analysis of serum oxylipins showed higher levels of AA-derived LOX and CYP metabolites in mice fed the control diet. Hence, DHA feeding dampens the ECS, activation potential, fundamentally altering macronutrient metabolism and physiology. GM analysis of serum samples performed in young women (YW; 29.1 ± 4.2 y), older woman, (OW; 74.6 ± 6.4 y) and men (75.2 ± 9.8 y) showed that fatty acid oxidation markers and fatty acid levels were higher in older subjects. Acylcarnitines were elevated in OW compared to YW but did not differ between OW and men. Acetoacetate was higher in OW compared to older men. EPA, DPA, and DHA, were higher in older women compared to YW but did not differ from older men. Indicators of elevated mitochondrial beta-oxidation were higher in older subjects. In summary, the findings indicate that dietary n-3 PUFA are associated with changes in muscle which are beneficial to conditions of aging. Dietary n-3 PUFA resulted in changes in oxylipins, anabolic endocannabinoids, and glucose sensitivity in muscle which could benefit aging.

oral 089	30 min	epidemiology	Cornelia M. Ulrich

Metabolomics and Prospective Cohorts: How to Leverage Resources, including WHI-OS and ColoCare

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Metabolomics is becoming an increasingly powerful tool in cancer research (reviewed in Liesenfeld et al, Cancer Epidemiology, Biomarkers & Prevention 2013;22:2182-201). Our group contributes currently to research in cancer metabolomics and participates in the COMETS (COnsortium of METabolomics Studies) consortium with studies in two study populations: (1) the ColoCare Study, a multicenter patient cohort of colorectal cancer patients (stages I-IV) and (2) a nested case-control study of colorectal cancer within the Women's Health Initiative Observational Study cohort (WHI-OS). The ColoCare study is a multicenter, prospective cohort of colorectal cancer patients that aims to identify strategies and biomarkers for tertiary colorectal cancer prevention. Within ColoCare we have metabolically profiled blood (baseline) and urine (baseline and follow-ups), as well as paired visceral adipose and subcutaneous adipose tissue samples collected during surgery in various patient subsets. All biospecimen were metabolically profiled using (1) the Biomarkers Laboratory of the Division of Preventive Oncology, NCT, and (2) the NIH-West Coast Metabolomics Center, Davis. Additional metabolomics research in collaboration with IARC, Lyon, France, is underway. The WOMIn study (Women, One-Carbon Metabolism and Inflammation) is a case-control-study of 988 colorectal cancer patients and matched controls nested within the WHI-OS. Metabolomic profiling was performed at Metabolon. Results of these complementary metabolomic approaches and their first integration with other –omic strategies will be presented. Selected reference: Liesenfeld et al., Metabolomics 12/2014; DOI:10.1007/s11306-014-0758-3 Utilizing both case-control and patient-cohort settings we are advancing research on metabolomic biomarkers in colorectal cancer.

oral 090	15 min	epidemiology	Francesco Savorani

Forecasting individual breast cancer risk using plasma NMR-Metabolomics and biocontours

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Breast cancer is a major cause of death for women. To improve treatment, current oncology research focuses on discovering and validating new biomarkers for early detection of cancer; so far with limited success. NMR metabolic profiling of plasma samples and auxiliary lifestyle information was combined by chemometric data fusion. It was possible to create a biocontour, which we define as a complex pattern of relevant biological and phenotypic information. While single markers or known risk factors have close to no predictive value, the developed biocontour provides a forecast which,several years before diagnosis, is on par with how well most current biomarkers can diagnose current cancer. In the current research work, samples from 838 Danish women enrolled in the Danish Diet, Cancer and Health cohort have been analyzed. Half of the women were diagnosed with breast cancer between time of enrollment and the chosen follow-up date. Plasma samples were withdrawn in a non-fasting state and analyzed by proton NMR. NMR spectra were transformed into a less redundant metabolic representation by using integrals of 129 identified spectral regions. In addition, 47 variables were added containing information about the lifestyle and phenotype of the subjects. All models were built using the chemometric classification model, Partial Least Squares

Discriminant Analysis (PLS-DA). Variable selection by forward selection was used to further improve the classification model always applying rigorous validation. The best PLS-DA classification model obtained uses a total of 27 of the original variables. The resulting model provides a hitherto unseen effective means for forecasting breast cancer with an error of 18%. A model based only on NMR was also evaluated and led to a model with a classification error of 22%. Hence, the NMR part of the data by far contains the most important part of the information. In the obtained classification models, it was investigated if any one variable was crucial for the classification, but this was not the case. Instead, it is the pattern of biological data—a biocontour—which is required for accurately predicting the risk. In fact, any of the variables may be substituted without major loss of predictive power indicating substantial informational redundancy in the data set. While e.g. mammography can diagnose current cancer with a sensitivity and specificity of around 75%, the currently developed biocontour can predict that there is an increased risk that breast cancer will develop in a subject 2–5 years after the sample is taken with sensitivity and specificity well above 80%. The model was built on data obtained in 1993–1996 and tested on persons sampled a year later in 1997. Metabolic forecasting of cancer by biocontours opens new possibilities for early prediction of individual cancer risk and thus for efficient screening. This may provide new avenues for research into disease mechanisms. Metabolomics enables forecasting breast cancer 2-5 years before diagnosis by means of biocontours (patterns of relevant biological and phenotypic information)

oral 091	15 min	epidemiology	Saskia Decuypere

Innovating diagnosis of bacterial bloodstream infections in malaria-endemic settings: from disease metabolomics to rapid diagnostic tests

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Bacterial bloodstream infections (bBSI) are increasingly recognized as an important cause of severe febrile illness in children in sub-Saharan Africa, with non-typhoidal Salmonella being the most frequently encountered pathogen causing 860,000 deaths each year. Currently, diagnosis of bBSI is clinical as microbiological culture testing is usually not available and if available, takes 2 to 3 days for a result. Missed diagnosis can result in preventable deaths, while overdiagnosis results in unnecessary use of antibiotics. There is an urgent need to develop rapid diagnostic tests targeting bBSI. We hypothesize that pathophysiological processes triggered by bBSI induce characteristic changes in the > 4,000 different blood metabolites. The objective of our study is to harness these characteristic metabolite features for bBSI diagnosis. We profiled plasma metabolites from 67 children (aged 2 months – 15 yrs) with severe febrile illness admitted to a district hospital in Burkina Faso using 3 different mass-spectrometry platforms: GC-TOF MS, C8-LC-QTOF MS, C18-UHPLC-LTQ Orbitrap MS. The patients included (i) 17 bBSI cases confirmed by blood culture or by deep sequencing, (ii) 34 severe malaria controls and (iii) 21 controls with negative diagnostics tests for bBSI and malaria. Following primary raw data processing, we used partial-least-squares regression analysis to examine to what extent the 30 documented patient/disease characteristics were reflected in the measured metabolome. We further mined the metabolome profiles for (a) potential individual bBSI biomarker compounds using ROC analysis and (b) bBSI biomarker signatures using supervised multivariate modeling. The three platforms yielded the following number of unique features that could be reproducibly quantified (feature RSD in pooled quality control samples 30 features that were fair bBSI classifiers (ROC area under curve > 0.75) with the majority present in higher concentrations in both Gram-negative and Gram-positive bBSI cases compared to the control groups. Most of these bBSI features are related to the host gluco/mineralo-corticoid response, which appears to be upregulated in bBSI patients but not in patients with similar symptoms due to severe malaria. A first bBSI diagnostic signature consisting of six features all sharing the steroid skeleton structure was found to have a sensitivity of 0.88 (95%CI: 0.71-1) and a specificity of 0.88 (95%: 0.78-0.96) which overall is a superior performance than the current gold standard of blood

culture. Mining of the metabolome data is ongoing to identify additional diagnostic signatures for differential diagnosis of bBSI and severe malaria. We will present the predicted diagnosis of all patients by the final metabolite diagnostic model(s), and compare to the results obtained with blood culture, deep sequencing and malaria diagnostic tests. This study demonstrates the potential of metabolite signatures to identify infectious causality in children with severe febrile illness.

oral 092	15 min	epidemiology	Beate Kamlage

A novel plasma based metabolic biomarker for the differentiation of pancreatic ductal adenocarcinoma carcinoma (PDAC) from chronic pancreatitis (CP)

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Pancreatic cancer (PDAC) is characterized by poor prognosis with 5-year survival rates of less than 5% and an increasing worldwide incidence (appr. 18/100.000). Chronic pancreatitis (CP) has a 26-fold higher risk for the development of pancreatic cancer. Established diagnostic methods such as transabdominal ultrasound and CA19-9 testing suffer from insufficient clinical performance. Therefore, the differential diagnosis of the both diseases remains a clinical challenge. Metanomics Health GmbH has developed a blood based metabolic biomarker for the differential diagnosis of PDAC vs CP with a sensitivity of 89.9% [95%CI 81.0-95.5] and a specificity of 91.3% [95%CI 82.8-96.4]. For a case-control study in three tertiary referral centres 914 subjects were prospectively recruited with either PDAC (n=271), CP (n=282), liver cirrhosis (n=100), or healthy as well as non-pancreatic-disease controls (n=261). An initial multicentre exploratory study (n=201) was followed by a second multicentre training study (n=474) and a third monocentric test study (n=239). Metabolomic profiles of plasma and serum samples were generated from 477 metabolites identified by gas-chromatography–mass spectrometry and liquid-chromatography-tandem mass spectrometry. Prior to statistical analysis, log10 transformation of ratios was conducted so that the data distribution becomes approximately normal. SIMCA-P version 13.0 (Umetrics AB, Umea, Sweden), TIBCO® Spotfire® 3.3.1 and R 2.8.1 were used for data analyses and visualizations. A biomarker signature (9 metabolites and additionally CA19-9 was identified for the differential diagnosis between PDAC and CP. The biomarker signature distinguished PDAC from CP in the training set with an area under the curve (AUC) of 0.96 [95%CI 0.93-0.98]. The biomarker signature cut-off of 0.384 at 85% fixed specificity showed a sensitivity of 94.9% [95%CI 87.0-97.0]. Using the same cut-off in the test set an AUC of 0.94 [95%CI 0.91-0.97] with a sensitivity of 89.9% [95%CI 81.0-95.5] and a specificity of 91.3% [95%CI 82.8-96.4] were achieved successfully validating the biomarker signature. In chronic pancreatitis patients with an increased risk for pancreatic cancer (incidence 1.95%) the performance of this biomarker signature results in a negative predictive value of 99.9% [95%CI 99.7-99.9] (training set) and 99.8% [95%CI 99.6-99.9] (test set) which could greatly aid physicians in optimizing treatment. A new metabolomics test for pancreatic cancer which has the potential to be used to screen patients at risk.

oral 093	15 min	epidemiology	Warwick Dunn

Severe Burn Injuries: Applying Metabolomics to Study Longitudinal Changes, and Prediction of clinical outcomes

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There are 11 million burn injuries globally each year, >300,000 deaths and significant healthcare costs (estimated £140million in the UK). The sequelae of severe thermal injury can include a hypermetabolic state, acute inflammation, immune dysfunction, sepsis, multiple organ failure (MOF) and death. The hypermetabolic response is shown to change longitudinally starting with a phenotype of insulin resistance and decreased metabolic rate followed by a second phenotype of increased oxygen consumption, increased heart rate and increased metabolic rate. The Scientific Investigation of Biological pathways Following Thermal Injury (SIFTI) Study in the UK is working to identify metabolic changes to be applied to derive new treatment options for hypermetabolism and to predict outcome and the later onset of sepsis and MOF. In the SIFTI study, longitudinal spot urine and serum samples were collected from day 1 to day 180 from >70 patients submitted to the Queen Elizabeth Hospital in Birmingham UK with burns greater than 15% Total Body Surface Area. Urine was diluted and analysed applying a HILIC UHPLC-MS method (Accucore 150-Amide HILIC, 100x2.1mm, 2.6 µm particle size; Ultimate U3000 and Q-Exactive mass spectrometer, Thermo Scientific). Serum was prepared by deproteinisation followed by analysis applying a reversed phase UHPLC-MS method (Hypersil Gold C18, 100 x 2.1mm, 1.9µm particle size, Ultimate U3000 and Q-Exactive mass spectrometer, Thermo Scientific). Data were processed (XCMS), metabolites were annotated (PUTMEDID_LCMS) followed by univariate, multivariate and correlation analysis. Urine and serum analysis showed two distinct metabolic phenotypes; phenotype one up to day 7 and phenotype 2 from day 7. Phenotype one identified 4 metabolites involved in the early stages of purine metabolism which were two-to-twenty fold greater at day 7 compared to day 1 (e.g. 4-ureido-5-imidazole carboxylate) and 3 metabolites involved in the glycolytic pathway which were two-to-fifteen fold greater at day 7 compared to day 1 (glycerate-diphosphate, phosphoenolpyruvate and lactate). Phenotype two showed a diverse range of metabolic changes including choline metabolism (choline, choline phosphate and choline sulphate were all increased at 90 days compared to day 7, purine metabolism (five metabolites all increased at day 90), folate metabolism (two metabolites increased greater than 2 fold at day 90), fatty acid synthesis (three metabolites increased 2-3 fold at day 90), aromatic amino acid metabolism (six tryptophan and tyrosine metabolites increased by >2.5 fold at day 90) and nicotinamide (two metabolites increased greater than 2 fold) metabolism. All metabolites discussed showed a statistically significant change ($p<0.05$) applying the Benjamini–Hochberg procedure. Metabolic signatures prior to sepsis and which could be applied to predict the later onset of sepsis were identified, though further validation in a larger and independent study is required. No statistically significant metabolic changes were observed in day 1 samples which could predict outcome (survival or death). The results show that metabolism plays an important role in post-injury shock and recovery and has the potential to predict the later onset of sepsis and provide patient management options to reduce complications. First longitudinal UHPLC-MS study burns patients' urine and serum to define metabolic changes during recovery and associated complications.

oral 094	30 min	Repair biochemistry + novel pathways	Andrew Hanson

Metabolite damage and its repair or pre-emption

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Two increasingly recognized facts about metabolism are that (i) metabolites are damaged by spontaneous and enzymatic side-reactions that form potentially toxic products, and (ii) cells have conserved enzymes that repair or pre-empt such damage. Identifying damage products and uncovering damage-repair and pre-emption enzymes are accordingly becoming frontiers in analytical, metabolic, and computational biochemistry. Some of the most iconic and universal cases of metabolite damage, repair, and pre-emption so far known come from B vitamin/cofactor metabolism. B vitamins and the corresponding cofactors are highly damage-prone, yet all cells must keep them intact because they are essential. The two following cases illustrate (i) the principles of metabolite damage/repair/pre-emption and (ii) how combining advanced analytical methods with comparative genomics and computational

approaches can drive discoveries in this field. (1) Repair of NADH and NADPH. These cofactors undergo enzymatic and chemical reactions that produce R and S forms of NAD(P)H hydrates [NAD(P)HX], which can inhibit dehydrogenases. The hydrates are repaired by the tandem action of an ATP- or ADP-dependent dehydratase that converts (S)-NAD(P)HX to NAD(P)H and an epimerase that interconverts the R and S forms. In plants, the dehydratase and epimerase are triple-targeted to plastids, mitochondria, and cytosol, enabling them to reconvert NAD(P)HX to NAD(P)H throughout the cell. (2) Pre-empting damage in riboflavin biosynthesis. The first two intermediates of riboflavin biosynthesis in plants and bacteria are reactive potential damage agents, and may be overproduced because the biosynthetic enzymes lack feedback controls. Excesses of these intermediates are disposed of by COG3236, a protein that is fused to different riboflavin pathway enzymes in plants and bacteria. COG3236 cleaves the N-glycosidic bond of riboflavin intermediates, yielding fairly harmless products and thus pre-empting damage to the riboflavin pathway. These repair and pre-emption exemplars are conserved across kingdoms of life. Such conservation is probably common among metabolite repair/pre-emption systems.

oral 095	15 min	Repair biochemistry + novel pathways	Daniel C Sevin *PhD student

Proteome-wide in vitro analysis of enzyme promiscuity in Escherichia coli using nontargeted metabolomics

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Catalyzing biochemical reactions with high specificity has traditionally been considered a hallmark of metabolic enzymes, which are thought to have evolved from catalytically inefficient and unspecific ancestors. Accumulating evidence, however, suggests that many extant enzymes have retained their ability to act on multiple substrates or catalyze different reactions at physiologically relevant rates. Here, we report our results of testing all known metabolic enzymes in Escherichia coli for catalytic activity towards hundreds of potential substrates by incubating them in a mixture of complex metabolome extracts. Our data reveal that enzyme promiscuity extends far beyond what is currently appreciated, and suggest that promiscuous enzymes played a key role for organisms to acquire new metabolic capabilities when confronted with adaptation to changing environments. We expressed and purified 1,043 known metabolic enzymes from E. coli using the ASKA collection. Each enzyme was separately incubated in a complex metabolome cocktail consisting of combined cellular extracts of Escherichia coli grown in different nutrient combinations supplemented with general enzyme cofactors. Over the time course of 15 minutes, aliquots of each reaction mixture were sampled and quenched. A total of 13,000 samples were analyzed using nontargeted flow-injection time-of-flight mass spectrometry, revealing temporal dynamics of 10,000 detected ions annotated as up to 1,500 metabolites based on accurate mass. Discovered promiscuous reactions were integrated into a genome-scale metabolic model of E. coli to investigate their contribution to the adaptability of metabolism to changing nutrient availability in the environment. For 60% of the analyzed enzymes we detected ions depleting or accumulating over time, corresponding to substrates and products of catalyzed biochemical reactions. Among these active enzymes, 70% affected at least one ion that did not correspond to any of their previously known reactants, suggesting promiscuous activity. By comparing the molecular structures of annotated novel reactants with known reactants, we noted that for some enzymes the structures were highly similar, consistent with the concept of “substrate promiscuity” according to which enzymes can catalyze the same biochemical reaction with closely related substrates. In other cases, novel and previously known reactants were structurally dissimilar, suggesting that these enzymes were able to catalyze mechanistically different reactions (also referred to as “reaction promiscuity”). The newly discovered promiscuous activities of several enzymes were (and are being) validated using enzyme assays and deletion mutants. Analyzing potential roles of this wide-spread promiscuity on metabolism within a genome-scale metabolic model suggest that promiscuous enzymes expand the metabolic capabilities in adapting to novel environments (i.e. accessing novel nutrient sources). We will further discuss the implications of wide-spread enzyme promiscuity for biotechnology and medical research. We present the first global enzyme activity screen, revealing unprecedented insights into the unexpectedly diverse catalytic capabilities of metabolic enzymes.

oral 096	15 min	Repair biochemistry + novel pathways	Markus Keller * Early Career Scientist

The role of non-enzymatic metabolic reactions during origin and evolution of metabolism and their impact on modern metabolic networks.

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The metabolic network is central part of every living organism and required for providing building blocks for the synthesis of proteins, lipids and DNA. Although there are variable enzymatic solutions to establish this network, the underlying chemical routes are surprisingly similar. Recently, we were able to identify a cascade of non-enzymatic reactions that is highly superimposable with modern metabolic routes (Glycolysis and pentose phosphate pathway) and that is strongly accelerated and increases in specificity by conditions resembling the ferruginous Archean Oceans (about 3.5 – 4 billion years ago). This suggested that at least parts of the metabolic structure were already present at the emergence of first live and that first organisms potentially exploited and sequentially integrated into enzymatic metabolism. Using quantitative liquid chromatography tandem mass spectrometry (Agilent 6460 Triple Quadrupole LC/MS), we monitored chemical reactivity of metabolic intermediates in a comprehensive simulation of early Archean oceanic environments. The targeted nature of our approach allowed absolute quantification of intermediates formed under these conditions and to calculate intermediated stabilities, reaction specificities and reaction rates. Our data reveals that the Archean ocean composition shaped metabolic evolution by stabilizing key moieties such as ribose 5-phosphate, and by facilitating specific interconversions among complex pre-biotic sugars phosphates. We find that this principle is not only restricted to sugar phosphates, but can also be extended to other central metabolite classes. In systematic perturbation experiments the non-enzymatic flux through different parts of the network could be modulated and gave rise to previously “silent”, specific reactions routes, allowing us to reconstruct the roles of non-enzymatic reactions during origin and evolution of metabolism. These observations also impact on other fields in metabolism research: Non-enzymatic reactions are still important part of the metabolic network as the presence of an enzyme does not prevent parallel non-enzymatic reactions to occur. Due to the lack of systematic data, metabolic modelling approaches often neglect non-enzymatic reactions. However, they are of relevance for organisms, especially when exposed to harsh environmental conditions (temperature/radiation). Non-enzymatic reactions also constrain the selection of novel drug targets. At best a coexisting non-enzymatic route limits drugs efficiency. As many enzymes function to prevent undesirable side-reactions (negative catalysis), an increased non-enzymatic contribution could cause adverse side-effects. Intriguingly, this principle could be exploited to challenge target cells, as for example cancer cells or bacterial pathogens, with high levels of toxic metabolites. Non-enzymatic reactions profoundly shape metabolism and consequently impact on metabolic modelling approaches and drug-design.

oral 097	15 min	Repair biochemistry + novel pathways	Carole Linster

Enzyme function discovery in the post-genomic era

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Thousands of genes in sequenced genomes encode proteins of unknown function. A significant fraction of those proteins are predicted to have enzymatic activities. This is in apparent contradiction with well-established metabolic networks in which a majority of reactions are now genetically identified. Recent studies indicate that the remaining putative enzymes don’t have redundant or physiologically irrelevant roles, but rather carry novel functions involved for example in metabolite damage control. I will describe how we combined comparative genomics, traditional biochemical and MS-based methods to discover new enzymes acting on by-products of normal metabolism. I will also present a more systematic enzyme discovery pipeline involving in silico functional predictions and non-targeted metabolomics analyses that we started to successfully implement in the lab. The hypothesis-driven enzyme function

discoveries involved classical biochemical methods such as recombinant protein production, protein purifications, and enzymatic activity assays. Targeted GC- and LC-MS-based approaches as well as gene silencing methods were used to demonstrate that the enzyme activities identified in vitro represent also the physiological role of the enzymes inside of the cell. A pipeline for more systematic enzyme function discoveries is currently being set up and validated using Saccharomyces cerevisiae. It involves in silico functional predictions based on Hidden Markov Models generated in-house using metabolic databases. Non-targeted GC- and LC-MS-based metabolomics analyses of wild-type versus putative enzyme knockout strains are used to identify endogenous substrate candidates. Functional hypotheses generated in this way are validated on recombinant protein level. The identification of the molecular mechanisms involved in the development of L-2-hydroxyglutaric aciduria, a severe neurometabolic disease, is at the basis of the metabolite repair concept. This disease is caused by the deficiency of an enzyme that specifically degrades L-2-hydroxyglutaric acid, a toxic side-product formed by malate dehydrogenase. Several other metabolite repair enzymes have been described since and I will explain how we identified some of them (GDP-glucose phosphorylase, NADHX dehydratase and epimerase, ethylmalonyl-CoA decarboxylase). While these results have recently been published, I will also show non-published data on the characterization of cells deficient in certain metabolite repair enzymes. Concerning the more systematic enzyme discovery pipeline, our HMM-based bioinformatics approach has allowed to classify the 1500 yeast proteins of unknown function into distinct functional categories (e.g. enzymes, transporters, transcription factors). Approximately a third of those proteins are predicted to have catalytic activities, with about 250 of those putative enzymes acting on metabolites. Using priority criteria such as conservation in humans and link to disease, these bioinformatics analyses allowed us to shortlist about 30 yeast proteins for further investigation. Non-targeted metabolomics experiments were performed to test whether the comparative analysis of metabolite extracts of wild-type yeast strains and yeast strains with enzyme gene deletions allows us to identify the endogenous substrates of the silenced enzymes. Using yeast knockout strains for known TCA cycle enzymes, we could indeed identify significant accumulations of the expected enzyme substrates in the knockout strains using a non-targeted GC-MS method. The analysis of a yeast knockout strain for one of our priority proteins of unknown function by non-targeted metabolomics experiments on both GC-MS and LC-MS instruments allowed the identification of its endogenous substrate and subsequently, the identification of the enzymatic activity and probable metabolic role of this yeast protein, as well as of its human homolog. Combining biochemical expertise with comparative genomics and metabolomics to more systematically unravel new enzyme functions in underinvestigated aspects of metabolism.

oral 098	15 min	Repair biochemistry + novel pathways	Gary Patti

Can 2-deoxyglucose be metabolized? An isotope-based metabolomic analysis

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2-deoxyglucose (DG), or [18F]FDG, is a radiopharmaceutical used in medical imaging for positron emission tomography (PET). [18F]FDG serves as a marker for glucose uptake. Like glucose, DG is taken up into cells. It is generally accepted, however, that DG is a competitive inhibitor of glycolysis and therefore is not extensively metabolized. Cancer tissues, which are undergoing aerobic glycolysis, take up increased amounts of glucose relative to most other tissues. [18F]FDG imaging is therefore the standard test used in cancer diagnosis and patient management. The objective of our work is to determine if DG is metabolized into other compounds. Metabolism of [18F]FDG to other [18F]-metabolites could complicate the interpretation of PET results and may offer an opportunity to phenotype cancer patients. HeLa cells (n=3) were incubated with labeled DG in parallel with the corresponding unlabeled metabolite for various time courses, then harvested in ice-cold methanol. The experimental conditions and harvesting protocol were kept identical between sample groups. After extracting metabolites from the cells in methanol, acetonitrile, and water, samples were run on a 6540 Agilent QTOF using HPLC LC/MS with a HILIC column in both positive and negative mode. Data analysis was performed using XCMS Online and isoXCMS (available as an R package). The experiment was also performed by giving mice labeled DG and analyzing their muscle tissue. Both HeLa cells and muscle tissue were analyzed by both LC/MS and solid-state NMR. After feeding cells 13C labeled DG, approximately 35 labeled compounds were detected using isoXCMS. isoXCMS pairs peaks that have similar retention times and m/z values that correspond to isotopologues. To putatively identify these potential isotopologues, the isotope database isoMETLIN was utilized. isoMETLIN has hundreds of experimental isotopomer MS/MS spectra. Alongside isoXCMS, XCMS Online was also used to compare the metabolic perturbation between cells with added unlabeled DG and cells that did not receive additional DG to ensure that no major changes

were being observed. Given that the LC/MS peaks found to be labeled represent an unknown amount of carbon, we performed total accounting of 13C label by using solid-state NMR. These data indicate that 15-20% of the 13C labeled DG peaks detected have been metabolized to substrates with different chemical shifts. Based on these results, our current hypothesis in which DG is metabolized through the pentose phosphate pathway will be discussed. This is the first experiment examining DG metabolism with untargeted metabolomics and has potential implications for clinical cancer imaging.

oral 099	30 min	compound ID and databases	Antoni Aguilar-Mogas * Early Career Scientist

iMet: A new tool for the annotation of unknown metabolites

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Structural identification of metabolites in complex biological mixtures relies on tandem mass spectrometry (MS/MS) analysis. Unfortunately, MS/MS spectra of a large number of known metabolites are not described in databases. This makes annotation of metabolites much more challenging than for peptides or nucleic acids. There is not a general tool that allows structural annotation of completely unknown metabolites (metabolites that are not yet annotated in any database) from their MS/MS spectra. Here we present iMet, a computational tool that uses MS/MS spectra and the exact mass of an unknown metabolite to identify structurally similar metabolites found in databases, and suggests a net atomic addition or removal that converts the known structurally similar metabolite into the unknown one. To search for compounds in a given database that are structurally similar to the unknown metabolite, iMet uses a machine learning algorithm. Once trained with pairs of known metabolites taken from a database, it is able to recognize, from their MS/MS spectra, those metabolites that are structurally similar from those that are not. When confronted with an MS/MS spectra from an unknown metabolite, iMet computes the MS/MS spectral similarity between the unknown metabolite with each compound of the database, along with the mass difference computed between them, and produces a sorted list of candidates according to their similarity with the unknown metabolite. It also suggests the structural differences between the candidates and the unknwon metabolite. We performed cross-validation experiments using experimental MS/MS spectra as well as leave-one-out procedures, testing a wide variety of metabolites that included nucleotides and nucleosides, both natural and unnatural amino acids, vitamins, sphingolipids, polyamines and fatty acids, amongst others, to ensure structural and biochemical diversity of tests. All of the metabolites tested were excluded from the training set and manually removed their entries from our database, effectively turning them into unknown compounds for the purpose of validation. In these tests, iMet showed a remarkable stability and performance. After testing more than 150 individual unknown metabolites, iMet was able to correctly identify a structurally similar compound from the database as the top candidate in 78% of the cases. In 91% of the cases at least one of the top four candidates was structurally similar to the test metabolite. In 88% of the cases, the top formula proposed by iMet was the correct formula of the test metabolite. Our algorithm has proven itself to be an unique tool in the identification of unknown metabolites as a stand-alone application. But perhaps more importantly, it could have a huge potential when coupled to other applications such as MetFrag, MetFusion, MAGMa, CFM-ID, or MS2Analyzer, where the output of iMet could be used as inputs for such algorithms. Overall, iMet provides the conceptual and computational basis for metabolomics to evolve as fast as the other omic sciences.

oral 100	15 min	compound ID and databases	Gert Wohlgemuth

MassBank of North America: an open access metadata-centric, auto-curating repository for mass spectra from different instrument platforms

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MassBank of North America (MoNA) is a unique metadata centric repository designed for storing, categorizing, and curating mass spectra from all instrument platforms, independent of the method of acquisition. MoNA only requires

minimal metadata; namely, the InChI Key and actual spectra, while allowing many more metadata to be added using tags for user- defined queries of MoNA. MoNA stores all acquired data in a PostgreSQL relational database, while utilizing the Grails domain class model to present these relational data in form of objects and their associations. These objects can be accessed and modified by different services on the server, which are exposed as REST interface to the clients.

Data can be submitted by using either a simple wizard or spectra in the MSP, MGF, of Massbank format. To ensure uniqueness of the chemical compounds, we are utilizing the standard InChI key, which can be provided during the upload process or generated by the system by utilizing metadata such as the standard InChI Code, smiles, or even compound names using the Chemical Translation Service CTS2. Currently MoNA contains 180,000 different unique mass spectra. These were obtained by importing publically available experimental mass spectra libraries including MassBank and Fiehn BinBase experimental spectra, in addition to computationally predicted spectra, namely complex lipids from the LipidBlast library (136k spectra), manually enhanced by InChI keys and smiles codes. From these spectra, we calculated and indexed all unique available metadata fields and their values, as well as automatically correcting erroneous values and categorizing these spectra based on their metadata tags. This process resulted in 2.8 million queryable metadata values. Autocorrection and tagging is based on 56 different rules, starting from simple rules to determining if submitted spectra represent GCMS or LCMS spectra, to more complex rules that check if the provided derivative form of a compound compliant to the precursor ion data provided. Based on these rules, the system was able to classify all spectra into 11 different categories. Due to the utilized load balancer and the implemented algorithms, MoNA is able to store, curate, and validate about three spectra a second in the system for each utilized server.

To demonstrate the ease of interacting with MoNA, we are providing several implementations of the REST-based interface in the common programming languages Java, C#, and JavaScript. The JavaScript implementation has been developed as a complete AngularJS web application, and allows users to easily query and download spectra as well as uploading data in a guided approach. Automatically curating and storing >100k open access mass spectra, while providing users with an easy interface to interact with.

oral 101	15 min	compound ID and databases	Eoin Fahy

Metabolomics Workbench, an international data repository for metabolomics data and metadata, metabolite standards, protocols, analysis tools, tutorials and training

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Metabolite measurements are excellent readouts of organismal function and are often considered more representative of the functional state of a cell than other ‘omics' measures. In addition, many metabolites are conserved across animal species, facilitating the extrapolation of research findings in laboratory animals to humans. The Metabolomics Workbench (MW) is a part of the data coordinating effort of the NIH Common Funds Metabolomics project to provide data and tools from metabolomics projects to the broader research community. MW provides a computational platform to integrate, analyze, track, and disseminate large volumes of data from metabolomics studies, along with access to protocols and metabolite standards for metabolomic studies. We present our current and on-going work on MW to support Metabolomics research initiatives. The Metabolomics Program's Data Repository and Coordinating Center (DRCC), housed at the San Diego Supercomputer Center (SDSC), University of California, San Diego, has developed the Metabolomics Workbench (MW). The MW available on a cloud infrastructure is built on a multi-tier architecture involving databases, applications/tools and interfaces. The applications include query, search, up/download and statistical analysis and visualization tools. MW also provides the MWTab format for up/downloading data in a user-friendly format. In addition the metadata for all data presented in the MW is available on the MetabolomeXchange. The MW serves as a national and international repository for metabolomics data and metadata to provide analysis tools and access to metabolite standards, protocols, tutorials and training. The Metabolomics Workbench (MW) contains a repository of over 100 experimental metabolomics studies with data acquired by a variety of mass spectrometry and NMR techniques. A high-capacity data

infrastructure has been deployed to upload raw data, experimental protocols and processed results from institutions around the world. The website contains interfaces to browse, search, download and perform a wide range of statistical analyses on these data. The MW also contains a searchable database of over 60,000 metabolite structures with a classification system and links to a wide range of molecular resources. MW also provides the ability for the community to request metabolite standards and all synthesized standards are annotated in the MW. It is anticipated that the MW will serve as the most comprehensive resource for NIH metabolomics projects and research. A unique online resource for deposition and display of metabolomics data representing a wide range of species

oral 102	15 min	compound ID and databases	James Jeffryes * PhD Student

MINEs: Open access databases of computationally predicted enzyme promiscuity products for untargeted metabolomics

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In spite of its great promise, metabolomics has proven difficult to execute in an untargeted and generalizable manner. Liquid ChromatographyMass Spectrometry has made it possible to gather data on thousands of intracellular chemicals. The bottleneck in this process is the identification of metabolites based on their spectral features, which currently depends on incomplete biochemical databases. The resulting bias towards rediscovery of known compounds limits the number of annotated features and ensures that new metabolites are seldom discovered in untargeted studies. We propose an approach called Metabolic In silico Network Expansions (MINEs) that expand curated biochemistry databases by considering the products of known enzymatic activities to address these challenges. We utilize an algorithm called the Biochemical Network Integrated Computational Explorer (BNICE) and expert-curated reaction rules based on the Enzyme Commission classification system to propose novel chemical structures and reactions. These rules have been demonstrated to reproduce a large fraction of known biochemical reactions and predict enzyme substrate promiscuity that was subsequently verified experimentally. 198 of these generalized chemical reaction rules were applied once to all compounds in KEGG, EcoCyc and the YMDB resulting in MINE databases of predicted products and chemical reactions. Compound and reaction data are stored as collections in a Mongo Database and are freely accessible for non-commercial use via user-friendly web-tools at http://minedatabase.mcs.anl.gov and developer-friendly APIs The MINE constructed from the Kyoto Encyclopedia of Genes and Genomes (KEGG) COMPOUND database contains over 571,000 compounds, of which 93% are not present in the PubChem database. However, these MINE compounds have on average higher structural similarity to Natural Products (NP) than compounds from KEGG or PubChem. The KEGG MINE was able to propose annotations for 98.6% of a set of 667 MassBank spectra, 14% more than KEGG alone. As a demonstration of the practical utility of MINE databases, we applied the MINE to annotate untargeted metabolomics data from an E. coli knockout study. 493 distinct MS features were extracted, 47 of which were identified following a traditional annotation workflow using NIST MSPepsearch; in contrast, the EcoCyc MINE database proposed candidates for 132 of the accurate masses when searching with 5 mDa precision and with [M+]+, [M+H]+, [M+Na]+ adducts. The resulting MINE candidates were consistent with 93% of the NIST MSPepsearch results. We selected a feature that did not match any E. coli metabolites but matched at least one of the novel metabolites proposed in the MINE by the BNICE method for further study. The EcoCyc MINE database returned one potential hit for this metabolite, a phosphoethanolamine lipid not present in any searched chemical structure databases, including ChemSpider (www.chemspider.com) and PubChem. LipidBlast was used to confirm that the MSMS fragmentation pattern is consistent with PE(32:1). Detection and verification of additional novel metabolites is ongoing. MINEs expand incomplete biochemical databases for improved feature identification and are freely available though a website and APIs

oral 103	15 min	compound ID and databases	Hiroshi Tsugawa * Early Career Scientist

MS-FINDER: Integrated Strategy for Structure Elucidation on LC-MS/MS by using Chemo- and Bioinformatics Resources

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Liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) is the preferred tool for untargeted metabolomics: detection, identification, and quantification of biomolecules. The main bottleneck in its discovery phase is the structure estimation of unknown metabolites. Since data-independent acquisition in combination with mass spectral deconvolution provides comprehensive MS/MS spectra for all precursor ions, we propose the following three-step approach. First, formula calculation predicts a molecular formula from both MS1 and MS/MS in combination with the seven golden rules and a database for neutral losses. Second, structure data that match with the formula are downloaded via the PubChem Rest Service, and are computationally fragmented with the Chemistry Development Kit to check consistency. Finally, the likelihood scores are calculated to rank candidate structures. Molecular formulas for MS1 are first filtered by the seven golden rules, and are checked for the consistency with the MS/MS fragments and their neutral losses. Each formula candidate is also verified whether it has been reported in existing metabolome databases (the program internally stores 42,028 formula). Candidate formulas are then ranked by the number of satisfied criteria being checked. The structure data files consistent with the candidate are retrieved from the PubChem Rest Service and from our internal 210,686 structure list. Our in-silico fragmenter simulates the alpha-cleavage of linear chains up to three chemical bonds with consideration of bond-dissociation energy. Multiple bonds (double-, triple-, or cycles) are modeled as penalized single bonds in which hydrogens are lost. MS-FINDER predicts molecular structures from their MS/MS spectra and is available on Windows OS (.NET Framework 4.0 or later). Its source code is written in the C# language. The goal of this program is to predict the molecular skeleton, i.e. the first block of InChIKey. We first tested the program by MassBank records consisting of C, H, O, N, P, and S measured by the following instrument types: APCI-IT-TOF, ESI-IT-TOF, ESI-IT-FT, and ESI-QTOF (1140 positive-ion records and 205 negative-ion records). The precursor- and product-mass errors are set to 0.001 Da and 0.01 Da, respectively. Top five molecular formula candidates were searched in the PubChem Compound Database. In positive mode, MS-FINDER predicted the correct structures (without stereochemistry) of 65.9% as the first candidate. The percentages of within the top three and the top ten were 84.0% and 90.1%, respectively. In negative mode, the program predicted the correct structures of 63.4% as the first candidate. The percentages of within the top three and ten were 72.7% and 78.5%, respectively. The first software program to predict molecular structures from experimental MS and MS/MS spectra that extensively utilize online databases

oral 104	30 min	diseases of the central nervous system	Rima Kaddurah-Daouk

A Systems Approach to the Study of Alzheimer's Disease and Its Progression

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Alzheimer’s disease (AD) is a national public health priority. Despite advances, clinical trials have not yielded therapies to prevent or slow disease progression with recent failures highlighting our incomplete knowledge of mechanisms. Accumulating evidence suggests the synaptic failure in AD is associated with dysregulation in multiple metabolic networks and that AD is not a singular condition but may be a combination of altered networks (Alzheimer Summit 2012 and 2015). A partnership between the metabolomics community and the Alzheimer’s Disease Neuroimaging Initiative (ADNI) provides an opportunity to build a metabolomics database on over 2000 AD subjects enrolled ADNI and means to link metabolomics genetics and imaging data to define at a systems level failures in AD and its progression. Non-targeted metabolomics (GCTOF) and lipidomics (multi-dimensional mass spectrometry-based shotgun lipidomics MDMS-SL), as well as targeted electrochemistry metabolomics (LCECA) platforms were

used to define initial metabolic signatures for mild cognitive impairment (MCI) and AD. Univariate, multivariate, pathway analysis and metabolic networks were used to define and connect metabolic changes in AD. Based on initial findings we have added an array of targeted metabolomics approaches to further investigate pathways implicated. Subjects enrolled in ADNI I (830) are being profiled to replicate initial findings and to define signatures for disease progression. With help of imaging data we are connecting central and peripheral metabolic changes. In addition metabolomics data combined with GWAS data is used to define metabotypes for genotypes implicated in AD. Metabolites and their ratios revealed changes within tryptophan, tyrosine, methionine, and purine metabolic pathways. Partial correlation network showed total tau most directly related to purine pathway metabolite xanthine and a tyrosine pathway metabolites; amyloid-beta (Ab42) was related directly to an unidentified metabolite and indirectly to 5-indole acetic acid (5HIAA) and methionine. We used stepwise logistic regression models with cross-validation to assess the ability of metabolite markers from two metabolomics platforms GCTOF and LCECA to discriminate between clinically diagnosed AD participants and cognitively normal controls (testing area under the curve: 0.70 and 0.96, respectively). Lipidomics analysis highlighted major changes in phosphatidylcholines (PC), ethanolamine plasmalogens (PlsEtn), and the sphingolipidome (SL) pointing to major changes in membrane structure and function. We will share initial findings from ADNI I study that are in line with recommendations we laid out in the Alzheimer Summit of 2015 as steps to meet challenge laid out by President Obama to treat or prevent AD by 2025. Metabolomics data and its integration with genomics and imaging data provides a unique systems approach to the study of AD.

oral 105	15 min	diseases of the central nervous system	John Newman

Metabolomic Investigation of Human Stress Response Phenotypes in a Controlled Feeding and Induced Stress Paradigm

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Psychological stress influences central nervous system (CNS) function and metabolic health, but the mechanisms linking stress and disease are unclear. Vulnerability and resilience to the negative neurological and metabolic health effects of stress may be reflected in metabolic stress responses. Thus variable changes in metabolism preceding and accompanying psychological stress may provide new clinical markers of CNS and metabolic disease risk. As stress hormones are also influenced by food consumption, the evaluation of metabolic changes in the context of a controlled dietary exposure can be anticipated to exacerbate stress associated metabolic differences between individuals. This study has used a broad panel of targeted metabolic profiles to explore the interactions between an individual's stress response and changes in the plasma metabolome. Participants were healthy, peri-menopausal women (n=41) aged 42-51yr with a body mass index of 25.9 ± 5.1 kg/m2. Three hours after a standard lunch volunteers participated in a mental stress task (Trier Social Stress Test, TSST). Stress response was measured as the TSST-associated salivary cortisol change. Blood samples were collected at 5 times via catheter before and after the standard lunch and TSST. Concentrations of non-esterified fatty acids and oxylipins, esterified fatty acids (ToFA) and oxylipins, endocannabinoids, amino acids, biogenic amines, lyso-phosphatidylcholines, phosphatidylcholines (PCs), and sphingomyelins (SMs) by a combination of GC/MS and UPLC-MS/MS. Results were normalized, partial least square regression (PLSR) models were constructed and compared against randomly permuted models, and models were simplified using regression filters. Of the 41 participants, 39 complete records were obtained. After normalization, 149 of the 1750 (8.5%) variables were significantly correlated with the TSST-induced change in salivary cortisol (delta-Cortisol). Variables from pre-lunch (21%), post-lunch/pre-stress (19%), 30min post-stress (8%), 60min post-stress (15%) and 90min post-stress (35%) were correlated to the delta-Cortisol. Pre-stress levels of non-esterified linoleate alcohols were negatively correlated with delta-Cortisol, while esterified concentrations of these compounds and the auto-oxidation marker 9-HETE were positively correlated with this index. Conversely, pre-TSST concentrations of free 18-carbon epoxides and diols were positively correlated with delta-Cortisol. During post-stress sampling, amino acids, in particular tyrosine, threonine, and lysine were positively associated while aspartate was negatively associated with D-Cortisol. At 90min post stress, metabolites in the esterified lipid pool (ToFA, PCs, SMs) dominated the correlative variables, with highly unsaturated and saturated species being positively and negatively correlated with delta-Cortisol, respectively. Late time point mid- and short-chain acycarnitines were also

positively associated with delta-Cortisol. In particular, the observed increase in propyonyl-carnitine is consistent with mitochondrial cataplerotic stress. Of the measured biogenic amines, putraciene was found to correlate to delta-Cortisol at 3 of 5 time points, also consistent with an oxidative stress condition. Using regression based feature selection and a permuted 30% training/test set split and, a robust 26 variable PLSR model was achieved (Q2 = 0.6±0.2 RMSEP =0.4±0.1; p<0.0001). The model contained variables from each measured metabolite class and were distributed across time points. Using pre-stress measures, free oxylipins alone provide a reasonable prediction of delta-Cortisol; however both pre- and post-lunch variables were required. Similarly, together 60 and 90min post-TSST (i.e. 90 and 120 min post meal) data yielded a modest model, where neither alone could. Results suggest that dynamic changes in lipid mobilization and oxidative stress induced by a meal are correlated with an individual's stress responsivity. Metabolic covariates of the magnitude of stress response are identified and indicate elevated oxidative stress.

oral 106	15 min	diseases of the central nervous system	Christophe Junot

Cerebrospinal fluid metabolomics highlights alteration of multiple metabolic pathways in patients with hepatic encephalopathy.

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Hepatic encephalopathy (HE) is a neurological complication of acute or chronic liver diseases. The proportion of cirrhotic patients developing HE is about 20%, and 60 to 80 % of cirrhotic patients exhibit cognitive disorders potentially related to moderate HE. HE is caused by increased ammonia levels, inflammation and altered blood-brain barrier (BBB) permeability. Outside the liver, ammonia can be metabolized into glutamine in muscle cells and in astrocytes of the central nervous system. High glutamine levels are responsible for astrocyte swelling and brain edema. However, the pathophysiological mechanism of HE remains poorly understood. This prevents the development of therapeutic strategies. Cerebrospinal fluid (CSF) metabolomics was used to identify dysfunction of metabolic pathways in cirrhotic patients suffering from HE. CSF samples were collected on 27 control patients without any proven neurological disease and 15 HE patients of the Pitié-Salpétrière hospital. Metabolomic analysis was performed using 2 complementary liquid chromatography methods and detection was achieved by using high resolution mass spectrometry. Automatic peak detection and integration were performed using the XCMS software package. Grouping of features was performed using CAMERA software. Annotation with public databases (KEGG, HMDB and Metlin) was performed on XCMS data sets with a homemade informatics tool developed in R language. Intensities of each variable present in the XCMS peaklists were normalized using the LOESS algorithm. At last, metabolites were identified by using our spectral database and MS/MS experiments. LC/MS methods and bioinformatic data processing tools led to the identification of 150 metabolites in CSF samples. Our results indicate that HE patients can be easily discriminated from controls on the basis of metabolic information. Concentrations of 100 metabolites were found to be altered in HE patients. Metabotypes of HE patients display alterations in several major metabolic pathways including methylation, neurotransmitters, ammonia and energy metabolism pathways. The involvement of some of these metabolites and pathways in the development of HE is already well documented. This is for example the case with increased of CSF concentrations of glutamine, which is the product of detoxification of ammonia. Increased CSF levels of methionine and derivatives, and also of aromatic amino-acids, have also been described and could be related to a dysfunction of blood brain barrier. Furthermore, our metabolomic approach brought new findings regarding energy metabolism, which has been mainly investigated using animal and cell culture models until now. Dramatic increases in carnitine and acyl derivatives were observed and may reflect an increase in β-oxidation of fatty acids to compensate for a lack of ATP production (which has been reported in animal models). Increased concentrations of acetylated derivatives could support this hypothesis. Such acetylated compounds could originate from the excess of acetyl-CoA produced by glycolysis and β-oxidation and not used by the Krebs cycle. Although it is usually believed that β-oxidation is marginal in the central nervous system, as opposed to what occurs in the muscle and in the liver, recent studies reported that β-oxidation accounts to up to 20% of brain energy

production. In conclusion, CSF metabolomic studies are suitable to simultaneously analyze multiple metabolic pathways in HE. Each of these pathways could be targeted by therapeutic strategies. First CSF metabolomic analysis of patients with hepatic encephalopathy. Metabotype of 100 metabolites that reflect alteration of multiple metabolic pathways.

oral 107	15 min	diseases of the central nervous system	Anna Wuolikainen

Multi-platform metabolite profiling of CSF and plasma from individually matched patients with sporadic amyotrophic lateral sclerosis, Parkinson's disease and controls.

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Amyotrophic lateral sclerosis (ALS) and Parkinson's disease (PD) are heterogeneous neurodegenerative diseases that share molecular pathologies such as oxidative stress, mitochondrial dysfunction and protein aggregation. There are currently no useful clinical markers predictive of onset or rate of progression in ALS or PD. Since there is no analytical method that can provide a complete coverage of the metabolome, complementary techniques increase the metabolite coverage and expand the number of metabolic pathways that can be screened for perturbations. We have sought to characterize shared and unique metabolite perturbations in ALS and PD compared to individually matched controls using multi-platform metabolomics. PD patients were included between 0-60 months after onset and ALS was stratified to include sporadic ALS patients. Cerebrospinal fluid (CSF) and plasma samples from individually age-, gender- and sampling-date matched patients (22 ALS and 22 PD patients and 28 controls) were analyzed using targeted and untargeted gas chromatography (GC) and ultra-high pressure liquid chromatography (UHPLC) mass spectrometry (MS). We applied constrained randomization of the run orders and used a novel method for multivariate analysis, orthogonal projections to latent structures of effect projections (OPLS-EP) to extract metabolite perturbations to distinguish between the diagnoses. Combining results from all MS platforms 144 metabolites were uniquely identified in CSF and 196 metabolites were uniquely identified in plasma. Ketoleucine, ornithine, galactitol, glucaric acid 1,4 lactone, ribofuranose and sorbitol were significantly increased in CSF and indole and proline in plasma of ALS and PD compared to the matched controls according to OPLS-EP models and Wilcoxon's test (95%). Candidate metabolite biomarkers for ALS identified in earlier metabolomics studies as well as in a contemporary NMR study were validated, including increased creatine and decreased creatinine in CSF. Additionally α-butyric acid was found to increase in CSF and plasma of ALS patients compared to controls and PD. Previous candidate metabolite biomarkers for PD were detected as significantly altered in this study including alanine and ketoleucine. Using a combined CSF and plasma metabolite model between ALS and controls followed by prediction of PD, the PD group was found to be more similar to the controls at baseline (0 months) however patients with sample from 12, 36 and 60 months after onset showed progression towards the ALS group, partly due to plasma perturbations in the kynurenine pathway, increased CSF α-butyric acid and increased CSF glucose. The results support the idea of neurodegenerative diseases sharing metabolite profiles and points towards dynamic changes over time with disease progression. This suggests that timing is highly important and that understanding changes over time could be an important link for extracting robust diagnostic metabolite profiles. Using the 'power of two' to study responses in the human metabolome towards onset and progression of neurodegenerative diseases.

oral 108	15 min	diseases of the central nervous system	Kefeng Li * Early Career Scientist

Metabolomic Analysis of Antipurinergic Treatment in the Fragile X (Fmr1 knockout) Mouse Model of Autism Spectrum Disorders

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Autism spectrum disorders (ASD) are known to be caused by both genetic and environmental factors. Nearly 30 metabolic pathway disturbances have been reported in children with ASD over the past 50 years. We developed an expanded LC-MS/MS platform for targeted metabolomics and a workflow that facilitate deep chemical phenotyping and data visualization in complex disease. We report the use of these methods in the successful treatment of autism-like features in the Fragile X mouse model using the antipurinergic drug suramin. Suramin (20 mg/kg intraperitoneally) or saline (5 µl/gram) was administered weekly to Fragile X (Fmr1 knockout) and FVB control mice for 16 weeks, starting at 9 weeks of age. At 25 weeks, lithium heparin plasma was collected by submandibular vein lancet under light isoflurane anesthesia for metabolomic analysis. After the addition of 310 commercial and custom synthesized stable isotope standards, plasma samples were extracted with 4 volumes of 50:50 acetonitrile:methanol at -20˚C, centrifuged, and the supernatants stored at -80˚C until LC-MS/MS analysis. Metabolites were resolved by hydrophilic interaction liquid chromatography (HILIC) on a Luna NH2 column, and analyzed by electrospray ionization and scheduled multiple reaction monitoring (sMRM) targeting 673 metabolites on an ABSCIEX 5500 QTRAP triple quadrupole mass spectrometer. Suramin treatment resulted in normalized social behavior and improvements in brain synaptic structure and protein expression. Suramin produced shifts in 20 of 60 metabolic pathways interrogated in the direction of controls. Seventeen of these pathways were previously reported as abnormal in human studies of ASD. These included purine, fatty acid oxidation, eicosanoid, ganglioside, phospholipid, microbiome, bile acid, and redox metabolism. We present methods for the use of variable importance in projection (VIP) scores from partial least squares discriminant analysis (PLSDA) to identify impacted biochemical pathways within a custom, in-house database of MRMs and pathways. These data were then used to create an information-rich visualization in the form of a wallchart-style biochemical pathway map implemented in the open-source network tool Cytoscape. We describe methods that help bridge the gap between growth of custom databases and shared tools for metabolomic data reporting.

oral 109	30 min	mQTL : metabolism and genetics	Karsten Suhre

Genetics meets Metabolomics: human metabolic individuality in biomedical and pharmaceutical research

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Over 100 years ago, Archibald Garrod conjectured that inborn errors of metabolism are "merely extreme examples of variations of chemical behavior which are probably everywhere present in minor degrees", and that this “chemical individuality [confers] predisposition to and immunities from the various mishaps which are spoken of as diseases". With the advent of genome-wide association studies (GWAS), Garrod's conjecture could be proven in many instances. By studying the association between genetic and metabolic variation in blood and urine samples of thousands of individuals, over 150 loci of genetically influenced metabotypes have been identified so far. In many cases, the biological functions of the genes that harbors these genetic variants match the biochemical properties of the associated metabolites, which sometimes even allows for the prediction of enzyme and transporter substrate specificities, or the identification of unknown metabolites. In combination with computational network approaches, an overall atlas of human metabolic individuality can be constructed, which allows to link genetic variance and environmental factors through metabolic phenotypes as intermediate traits to clinical outcomes, and thereby opens an avenue to a rational manipulation of human metabolism for therapeutic means. In this presentation I shall provide a review of recent advances in the field of GWAS with metabolomics with a focus on how metabolomics may be utilized in conjunction with genetics to understand complex biological systems. The study of genetic variance in human metabolism provides access to experiments conducted by nature on complex biological systems.

oral 110	15 min	mQTL : metabolism and genetics	Marc-Emmanuel Dumas

Bayesian Tissue mQTL Mapping Reveals the Genetic Architecture of Housekeeping Regulatory Mechanisms and Organ-Specific Differentiation of Metabotypes in the Rat.

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The genetic regulation of metabolism underpins a large number of complex cardiometabolic and neurodevelopmental disorders. Recent advances in sequencing and in metabolic phenotyping (ie metabotyping) technologies facilitate the development of systems genomics approaches for metabolic traits in organs and biofluids in metabotype Quantitative Locus (mQTL) mapping studies. However, the tissue-specific genetic determinants regulating metabolite abundance remain elusive. To gain insights into the genetic architecture regulating fine-grained tissue-specific metabotypes, we profiled the metabolome of 5 tissues from the panel of 29 BN-Lx/SHR recombinant inbred rat strains. Snap frozen tissues (heart, liver, aorta, white adipose, brain) collected from 29 RI rat strains were mixed with 1.5 mL of 1:1 water:methanol solution and homogenised to extract polar metabolites; pellets were then mixed with 1.5 mL of 3:1 Methyl tert-butyl ether: methanol solution to extract organic compounds. Untargeted metabolic profiling of polar and organic fractions was performed on Acquity UPLC system coupled to Xevo G2 Q-TOF mass spectrometer (Waters), using RP and HILIC columns. Raw data were processed in XC-MS for feature calling and outputs were filtered for peak quality (repeatability, linearity, S/N peak shape). Bayesian mQTL mapping was implemented using ESS+, curated protein-interaction networks were used to predict structural candidates from features, then confirmed by HRAM-MS (Orbitrap, Thermo). We profiled the metabolome and lipidome in heart, aorta, liver, white adipose tissue and brain using untargeted ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-MS) generating >38,335 traits and identified 7,287 abundant features in heart and liver, including 3,440 common features. i) We demonstrate that tissue metabolite abundance is highly heritable and mostly polygenic (median H2 ranging 0.33 to 0.46 in heart and liver). ii) We reveal the existence of tissue-specific genetic control of metabolite abundance, by identifying 589 mQTLs, 71% of which being tissue-specific. iii) Combining mQTL- and interactome-mapping strategies, we identified tissue-specific cis-acting mQTLs through shortest path minimisation across protein-protein interaction networks and observed direct connections between a gene involved in synthesis, degradation or transport of a metabolite for 9 loci, the majority of gene-metabolite associations corresponding to long-range connectivities across biomolecular networks. iv) Genetic hotspots regulating up to 200 metabotypes reveal the presence of cis-acting loci involving genes characterized by low substrate specificity, v) Finally, complex tissue metabotype / phenotype correlation networks predict treatment efficacy and highlight novel disease mechanisms. Altogether, these multi tissue profiles reveal the detailed genetic architecture regulating metabolic traits that are directly translatable into disease-related mechanisms.

oral 111	15 min	mQTL : metabolism and genetics	Sarah Cherkaoui * PhD student

Predicting the impact of disease-associated genes on metabolites : A systematic approach based on pathway mapping of mGWAS data.

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This project is part of a larger multidisciplinary study on personalized medicine, of which the ultimate goal is to predict drug response for patients with inflammatory bowel disease. The latter study includes functional genomics assays that will identify the biological impact of genes selected from disease risk loci and drive selection of clinical assays in plasma from patients, which include metabolomics. The goal of the current project is to develop a bioinformatics pipeline to objectively predict, given a list of genes as input, which metabolites should be targeted for metabolomics studies. Our working hypothesis is that genes, involved in reactions catalyzed by their encoded enzymes, will predominantly impact metabolites close to these reactions in metabolic pathways. In order to validate our working hypothesis, we propose to use association data from genome-wide association studies (GWAS) combined with metabolomics (mGWAS) to map and compute distances between associated genes and metabolites on a pathway

database. This includes the following steps. 1) Downloading pathways from a selected database and parsing them into simple graphs, with metabolites as nodes and genes as edges; and 2) computing distances between edges and nodes using developed functions whereby the distance is defined as the number of reactions between a gene's corresponding enzyme and a metabolite in metabolic pathways. Data visualization is subsequently achieved using heat coloured matrices and distribution plots. The utility of our approach is illustrated using association data of the most recent and complete mGWAS study by Shin et al. (Nature, 2014) – a study which provides high-resolution reference atlas of human genetic influences and their metabolic relationships measured in vivo in blood. Specifically, Shin et al. identified 299 SNP-metabolite associations, which were annotated by the authors to implicate 132 genes. For this proof-of-concept, we have selected KEGG pathway database and its overview of metabolism since it is the most complete map of metabolic pathways. Using this approach, 50/132 genes were mapped to KEGG overview of metabolism. However, numerous challenges were encountered when mapping the 529 metabolites measured. For example, 33% of these metabolites could not be identified and 33% of the remaining metabolites were not found in the database. A method is proposed to partially bridge this gap, resulting in 97/529 metabolites mapped to KEGG overview of metabolism. We could compute distance for 129/299 gene-metabolite associations. In order to verify that the computed distance for gene-metabolite associations is smaller than that obtained by chance, we have computed distance between all combinations of genes and metabolites measured. The mean distance computed was 8.27 for measured genes/metabolites vs. 1.25 for associated genes/metabolites, thereby supporting our working hypothesis. Taken together, the proposed approach adds quantitative measures to gene-metabolite associations. The metabolites associated to given genes were found to be close, but not necessarily part of the reaction catalyzed by the enzymes encoded by the genes. The proposed approach should enable a better understanding of how genetic variants impacts metabolism. For example, this approach could be used to gain insight into the relevance of mGWAS data and can serve as a basis to predict which metabolites should be targeted, giving a list a gene as input. A novel bioinformatics pipeline to predict the impact of genes on metabolites and objectively assess relevance of mGWAS association data.

oral 112	15 min	mQTL : metabolism and genetics	Johannes Raffler * PhD student

A genome-wide association study with NMR metabolomics identifies 15 genetic loci with hitherto unknown urinary metabolic trait associations

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Genome-wide association studies on metabolic phenotypes (mGWAS) uncovered a large number of genetic variants that influence human metabolism. Thus, these studies provide unique insights into the genetic contribution to metabolic individuality. This individuality can be observed in various layers, for instance in altered metabolic homeostasis in blood. Homeostasis is a well-investigated subject of mGWAS, and over 150 genetic loci influencing metabolite concentrations in blood were reported in the past. Another aspect of metabolic individuality is excretion and detoxification, which can be investigated best in urine being the body’s main excretion vehicle. However, only 4

mGWAS in urine were published so far, reporting 11 loci with genome-wide significantly associated urinary traits. Here, we report the largest mGWAS in urine to date. We recorded one-dimensional 1H NMR spectra of the urine samples of 3,861 SHIP-0 cohort subjects. We analyzed these spectra both in a targeted and non-targeted manner, thus implementing the two most common approaches of NMR-based metabolomics. For the targeted analysis, we manually quantified a set of 60 metabolites in the spectra. For the non-targeted analysis, we used the same spectra and reduced them to a set of 166 distinct features (chemical shifts). We tested the targeted and non-targeted metabolic traits as well as the pairwise ratios within each trait type for genetic associations. For the interpretation of the results, we applied evidence-based locus-to-gene mapping and automated assignment of metabolites to non-targeted metabolic traits. We identified 22 genetic loci that display genome-wide significant association signals ($P < 3.25\times10\text{-}12$) in SHIP-0 and replicate in the independent KORA F4 cohort (N = 1,691). Our study therefore more than doubles the number of known loci that influence urinary phenotypes. Of the 22 loci, only 12 associate significantly with both targeted and non-targeted metabolic traits. 3 loci display significant association signals with targeted traits only, whereas 7 loci only associate significantly with non-targeted traits. Thus, our study clearly benefits from the combination of targeted and non-targeted NMR metabolomics analysis. 15 of the 22 uncovered loci were not reported in any previous urine mGWAS. These newly identified loci also host variants that were previously linked to chronic kidney disease, pulmonary hypertension, and ischemic stroke. By establishing connections from gene to disease via metabolic traits, our results provide novel hypotheses about molecular mechanisms involved in the etiology of diseases. Furthermore, a comparison with results from previous mGWAS shows that 14 of the 22 urinary loci are also associated with blood metabolites. This large overlap between loci influencing metabolic traits in urine and blood helps to elucidate the relation between blood homeostasis and its regulation through excretion. For most loci where the associations target the same or a closely related metabolite in both urine and blood, we observe the direction of the genetic effect to be consistent in both fluids. However, we find that one locus hosting a gene coding for a myo-inositol transporter is associated with elevated myo-inositol levels in urine, whereas previous studies report associations with decreased myo-inositol concentrations in blood. We conclude that this might be indicative for an altered renal reabsorption of this metabolite caused by genetic variants in this locus. We increase the number of genetic loci with significantly associated metabolic traits in urine from 11 to 26.

oral 113	15 min	mQTL : metabolism and genetics	Laura K. Reed

Quantitative trait locus mapping of genotype-by-diet interactions for metabolomic profiles underlying Metabolic Syndrome in Drosophila.

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Diseases linked to Metabolic Syndrome (MetS) such at type-2 diabetes and cardiovascular disease are rapidly increasing due to the influences of a modern Westernized-life style, but the genetic, environmental, and physiological mechanisms linking the symptoms of MetS remain to be elucidated. Large-scale studies to systematically assess how genotype interacts with the environment to cause complex disease are very difficult in humans, but such studies are relatively tractable in genetic model systems such as Drosophila melanogaster. Drosophila bears great homology to mammals in many aspects of relevant physiology and organ systems including insulin signaling, heart function, innate immunity, and response to aerobic exercise. In this study, we used the Drosophila Synthetic Population Resource (DSPR) derived from eight diverse founder strains to perform quantitative trait locus (QTL) mapping of MetS-like symptoms and metabolites across two dietary treatments (normal and high fat). Recombinant inbred lines of flies were crossed to restore heterozygosity, producing 390 distinct genotypes, and then raised from 1st through 3rd larval instar stages on each of the two dietary treatments. Third instar larvae were harvested and flash frozen for phenotyping and metabolomic profiling. Metabolomic profiles were performed at the West Coast Metabolomics Center at UC Davis using GC-TOF MS. QTL mapping was performed using a tailored computational pipeline developed at North Carolina State University. Previously, we have shown that that there is a very substantial contribution of genotype-by-environment interactions to the phenotypic variation observed for MetS-like symptoms (e.g. body weight, triglyceride storage, blood sugar, gene expression, and metabolomic profiles) in a naturally genetically variable population of D. melanogaster. We demonstrated clear correlations between metabolomic and gene expression profiles and MetS-like symptoms as they vary across diet. In this study, metabolomic profiling characterized at total of 421 metabolites (167 with confirmed chemical identification). We mapped multiple genetic loci that control main genetic variance effects on metabolite levels (independent of dietary influences) as well as genetic loci that specifically interacted with diet to influence metabolite profiles. Surprisingly, often the main genetic effect loci are entirely distinct from those that mediate genotype-by-diet interactions. Some genetic loci affect many metabolites, likely high-level metabolic regulators (analogous to trans-eQTLs), while others are more specialized

(analogous to cis-eQTLs). Genes within these loci provide both familiar and novel candidate genes for involvement in Metabolic Syndrome. In addition, many of the uncharacterized metabolites are highly correlated with MetS-like phenotypes indicating the pressing need for molecular characterization and confirmation of previously unidentified metabolites. Overall, this study demonstrates the power of model systems to leverage experimental tractability coupled with metabolomics methods to elucidate complex disease pathways. To our knowledge, this is the first genetic mapping study of genotype-by-environment interaction effects on metabolomic profiles.

oral 114	30 min	metabolomics methods	Tomoyoshi Soga

CE-MS metabolomics and application to cancer cell metabolism

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Cancer cells predominantly use the glycolytic pathway for the production of precursors for cell building blocks as well as ATP. This metabolic shift termed “Warburg effect” is now considered a fundamental hallmark of cancer. However, little is known about what induces alterations in cancer metabolism. Metabolomics has become a powerful tool for gaining insight into cellular and physiological responses. In 2003, we first reported capillary electrophoresis mass spectrometry (CE-MS) based metabolomic profiling and then a more high-throughput method based on CE-TOFMS. Metabolites are first separated by CE based on their charge and size and then selectively detected at their exact mass molecular ions. In this marriage of techniques, CE confers rapid analysis and efficient resolution, and MS provides high selectivity and sensitivity. The major advantages of CE-MS are that thousands of charged metabolites can be determined by two, cationic and anionic, methods. Recently this approach has been commonly applied to various fields of science. To understand the underlying mechanism of metabolic reprogramming of cancer cell metabolism, we applied CE-TOFMS metabolomics to tumor tissues and normal counterparts obtained from colon cancer patients. Quantification of hundred metabolites resulted in the identification of cancer-specific metabolic traits. Metabolome and transcriptome data showed enhanced glycolysis, pentose phosphate pathway, fatty acid synthesis and reduced Krebs cycle and fatty acid oxidation were found in most tumor tissues. Further, other interesting characterizations of cancer metabolism by omics analysis will also be discussed.

oral 115	15 min	metabolomics methods	Karl Burgess

Application of a novel GC-Orbitrap instrument to untargeted metabolomics to detect biomarkers for time of death in a rat model

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Time of death estimation is one of the most critical yet difficult tasks in forensic investigation. Current methods to determine time-of-death are inaccurate and based on visual inspection of the body. A laboratory-based method, using a robust biomarker for time of death would revolutionise forensic investigation. GC provides excellent chromatographic separation capability for biomarker discovery using untargeted metabolomics, but has previously been hampered by the lack of high-end mass spectrometry support providing the dynamic range, accurate mass and scan rate sufficient to analyse very complex samples such as mammalian muscle tissue. Here we describe the application of a complete untargeted metabolomics workflow using a novel GC-Orbitrap mass spectrometry system to detect biomarkers for time of death in a rat model. Pure standards mixtures were prepared in water and ethanol solvents. Rat thigh muscle tissue sections were dissected from an individual rat post-mortem at increasing time of decomposition. Metabolite extractions were performed by homogenising tissue sections with chloroform / methanol / water (1:3:1) and incubation for 1 hour over ice. Protein and DNA pellets were formed by centrifugation. The supernatant was removed and stored at -80C until required. Two-step derivatization was performed using O-methoxyamine HCl, followed by silylation with MSTFA. Automated derivatization and sample injection was performed using Thermo Fisher Triplus RSH, into a Thermo Fisher 1310 gas chromatograph. Analytes were separated using a Thermo Fisher TraceGold 5MS capillary column coupled to an Orbitrap system with electron ionisation interface. We describe an optimised workflow for metabolomics analysis on a novel GC-Orbitrap instrument [1], comprising sample

preparation with automated derivatization, an optimised GC gradient, spectral deconvolution of relevant peaks, identification via EI spectral matching, quantification using both multivariate and univariate statistics and contextualisation using biochemical pathways. We demonstrate this in a forensic study to determine biomarkers for time of death in a rat model. Performance was tested using three panels of isomer-separated standards. They were analysed using a 36 minute temperature gradient and demonstrated excellent chromatographic resolution and mass accuracy. Scan rate was sufficient to provide peak definition ideal for quantitation. EI fragment patterns were sufficient to provide high confidence IDs from the standard NIST libraries, while accurate mass fragmentation was capable of providing additional structural information for poorly characterised species. Biological samples (4 time points of rat muscle tissue) were analysed using the same 36 minute gradient with automated two-stage derivatization. Statistics applied to the datasets highlighted significant linear changes in amino acid concentrations over the period of decomposition. Absolute quantitation of amino acids was performed to accurately describe concentration changes and provide a reference range for the forensic laboratory. Complementary proteomics data was also generated using two-dimensional gel electrophoresis and mass spectrometry, demonstrating degradation of key muscle proteins, which may provide correlating evidence that the breakdown of protein species contributes to the changes in amino acid levels over the period of decomposition. Further research in collaboration with the Forensic Anthropology Center at Texas State University (San Marcos Texas) will repeat these experiments in human tissue. We describe potential impacts to the field of forensics. The developed method could also be translated for use in other fields such as the study of beef maturation. Reference: 1. Peterson, A. C. et al. Anal Chem. 2014 Oct 21;86(20):10044-51. Application of a novel analytical instrument to untargeted metabolomics. Application of metabolomics to ‘post-mortomics’. Biochemical time of death.

oral 116	15 min	metabolomics methods	John A. McLean

Targeting the untargeted: Structural mass spectrometry for the analysis of complex samples in systems, synthetic, and chemical biology

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One primary challenge in comprehensive analyses is the broad-scale characterization of the molecular inventory in cells, tissues, and biological fluids. Advances in computational systems biology rely on the experimental capacity to make panomics measurements, i.e. integrated metabolomics, proteomics, lipidomics, glycomics, etc. Ion mobility-mass spectrometry (IM-MS) provides rapid (ms) gas-phase separations on the basis of molecular structure and identification on the basis of high mass accuracy MS. This report will describe recent advances in IM-MS integrated omics measurement strategies in the analyses of complex biological samples of interest in systems, synthetic, and chemical biology. Advances in bioinformatics and biostatistics will also be described for unbiased and untargeted systems queries and to quickly mine the data for both targeted and discovery information. Samples such as cellular secretions, serum, whole tissue, and microbial extracts are analyzed using a combination of direct infusion, UPLC, GC, or SFC integrated with high performance IM-MS. For systems and synthetic biology studies, these entail integration of microfluidic devices ranging from cell-trapping platforms for exometabolome analyses to synthetic 3D human organotypic organs prior to the analysis stage commensurate with human-on-a-chip discovery strategies. Studies in chemical biology center on untargetted natural product discovery from microbial extracts prepared in the presence and absence of competing microorganisms and different cuturing conditions to encourage the production of unique secondary metabolites. Multivariate statistical analysis methods were utilized for prioritization of compounds for further targeting and identification. Our studies in high temporal resolution are motivated by characterizing metabolomics and metabolic profiling to provide sequential snapshots of cell and tissue physiology. While considerable effort has been applied to interpreting metabolic profiles to assess phenotype, these studies follow alterations to these molecular signatures in response to chemical and environmental perturbations. This work delineates the progress towards an online platform capable of performing integrated systems biology research using a combination of microfluidics, online desalting techniques, and ion mobility-mass spectrometry detection capabilities to provide dynamic exometabolomic sampling of biological systems. The exometabolome is rich in cellular and population conditional information and can indeed itself be highly indicative of intercellular processes. By virtue of utilizing fast (ca. ms) structural separations in combination with MS detection, we have demonstrated a means for delineating information from complex sample matrices which may be comprised of multiple biomolecular classes. Temporal resolution is demonstrated for model cellular systems and whole tissues in vivo including human Jurkat cell (T-cell lymphocytes), rat β cells, and bacteria, human-liver-on-a chip, among others. To elucidate the prevailing molecular signatures of phenotypic state, in an untargeted methodology, we use multivariate statistical methods and

self-organizing maps of molecular dynamics. The integration of these datasets reveals a more comprehensive perspective of the underlying systems biology, which combined with self-learning algorithms, yield heuristic models of dynamic biology. Broadscale strategy for untargeted and comprehensive metabolomics with high temporal resolution directly from biology of cells and tissues

oral 117	15 min	metabolomics methods	Zijuan Lai * PhD student

Annotation of novel metabolites in various biological systems using accurate mass GC/QTOF MS and validated fragmentation rules

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When profiling metabolomics samples by GC- electron ionization MS, spectral matching by NIST, Fiehnlib and MassBank libraries readily yields 100-150 identified metabolites per chromatogram. However, an even larger number of peaks remain unidentified. Unlike to LC-MS spectra, mass spectra in GC-MS show a great amount of fragment ions and a low abundance or absence of molecular ions. With chemical ionization and accurate mass GC-QTOF MS, molecular adduct ions can be obtained to calculate elemental compositions. We here utilize accurate mass GC-EI-QTOF MS spectra to generate fragmentation rules for diagnostic fragment ions, neutral losses, and ion series rations, and to apply those substructure assignment rules for annotation of novel metabolites in various interesting biological samples. 80 research articles from the 1960s to present were studied to accumulate legacy hypotheses on 572 fragmentation features with annotated substructures. In addition, 459 accurate mass electron-ionization spectra have been acquired for 309 chemical reference compounds using the Agilent 7200 accurate mass GC-QTOF MS. Fragmentation rules extracted from literature were validated based on both the NIST14 nominal mass library and the GC-QTOF MS accurate mass library. Subsequently, these fragmentation rules were applied to annotate potential structures of selected unknowns from various biological samples using stable isotope reagents analyzed under positive chemical ionization in addition to electron ionization. Chemical formulas were calculated and searched against MINE database. Candidate structures were obtained and validated by MassFrontier in-silico fragmentation prediction. Characteristic fragmentation patterns were generated for typical metabolites classes including alcohols, amino acids, carboxylic acids and tested for validity using accurate mass GC-QTOF MS using both false positive and false negative assessments (specificities and sensitivities). 459 accurate mass electron ionization spectra were acquired for 309 chemical reference compounds under trimethylsilylation. 572 fragmentation features and rules were then tested for accuracy to correctly assign molecular substructures of which 207 rules yielded true positive hits within 4mDa mass accuracy. These rules were used in conjunction with workflows for identifications of unknown compounds using adduct ion identification, PubChem searches, substructure constraint applications and MassFrontier fragmentation predictions. Selected unknown compounds were investigated by substructure constraints of fragmentation rules. The top hits were obtained from a novel enzyme expansion database (MINE), and were validated by MassFrontier in-silico fragmentation prediction. The performance of the entire unknown identification methodology was tested by the analysis of known unknowns. Examples and specific workflows will be presented, detailing ways to score for likelihood of correct metabolite annotations through accurate mass GC-QTOF MS. Several unknown metabolites from Escherichia coli, Chlamydomonas reinhardtii, Artemisia douglasiana, and nutritional food samples were identified at very high likelihood. Unknown compounds were successfully annotated using validated fragmentation rules and substructure constraint workflows with accurate mass GC-QTOF MS.

oral 118	15 min	metabolomics methods	Philip Britz-McKibbin

Multiplexed Separations for Metabolomics: An Accelerated Data Workflow for Biomarker Discovery in Cystic Fibrosis

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High efficiency separations are needed for enhancing selectivity, quantitative performance and mass spectral quality when analyzing complex biological samples that also supports unknown metabolite identification. However, a major constraint in large-scale mass spectrometry (MS)-based metabolomic initiatives is the low sample throughput and

complicated data pre-processing associated with conventional separations. Herein we introduce an accelerated data workflow for biomarker discovery in metabolomics to identify biochemical signatures of cystic fibrosis (CF) from dried blood spots (DBS) when using multi-segment injection capillary electrophoresis-mass spectrometry (MSI-CE-MS). This multiplexed separation platform takes advantage of customizable serial injections to enhance sample throughput and quality assurance within a single separation while encoding information temporally for reliable screening or confirmatory testing of in-born errors of metabolism (IEM). A seven-segment injection format was used in MSI-CE-MS in order to enhance sample throughput and peak capacity without loss of information content due to ion suppression. An asymmetric signal pattern recognition method was developed when analyzing methanol-based DBS extracts using analyst-blinded proficiency samples from the CDC as related to six different IEMs, including CF. Three DBS specimens were analyzed in duplicate in a single run alongside a pooled/normal sample as quality control for unambiguous identification and quantification of known disease-specific markers of IEMs. Also, untargeted metabolite profiling was performed by MSI-CE-MS in order to discover biomarkers that differentiate affected CF patients from healthy controls when using complementary univariate and multivariate statistical methods. Population-based screening for CF in North America currently relies on a two-tiered biochemical screen that suffers from high rates of false-positives and asymptomatic carrier identification that presents ethical concerns regarding widespread genetic testing. In this work, optimization of extraction conditions from cut-outs from DBS paper filter cards were first examined in order to maximize metabolite recovery for a wide range of polar/ionic metabolites with good precision. Method validation of MSI-CE-MS was then conducted based on targeted analysis on known biomarkers from DBS extracts for five IEMs associated with representative amino acid, fatty acid and organic acid disorders, including phenylketonuria, maple syrup urine disease, citrullinemia, medium-chain acyl-coenzyme A deficiency and glutaric acidemia-1. Acceptable precision and accuracy was demonstrated by MSI-CE-MS when comparing results for primary biomarkers for IEMs with expected CDC concentrations determined by MS/MS and cut-off value ranges used for newborn screening. MSI-CE-MS was also used as a high-throughput screening platform for biomarker discovery involving polar metabolites differentially expressed in CF patients homozygous for delF508. Over twenty-five metabolites in methanol-derived DBS extracts were found to be statistically different in CF relative to healthy controls using Bonferroni correction for multiple hypothesis testing. Unknown CF-specific metabolites were also identified when using MS/MS in conjunction with electrophoretic predictive modeling based on their putative chemical structure. MSI-CE-MS offers an unprecedented approach to increase sample throughput that is analogous to direct infusion-MS while offering greater selectivity, better quantitative performance, and higher data fidelity for biomarker discovery and unknown metabolite identification. These distinct analytical and bioinformatic merits are achieved without column switching, isotopic labeling, hardware modifications, or costly infrastructure investments. An accelerated data workflow and multiplexed screening approach is presented for identification of metabolite signatures of CF in DBS extracts.

oral 119	30 min	food & health	Joseph Rothwell

The polyphenol metabolome and its measurement in the in the European Prospective Investigation into Cancer and Nutrition (EPIC) study

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Dietary polyphenols are a complex group of about 500 compounds that are abundant in many plant food sources such as coffee, tea, fruits or wine. Some of these have been proposed to play a role in the prevention of cardiovascular diseases, diabetes and cancer. However, epidemiological evidence is often vague and contradictory due to difficulties in measuring exposure to polyphenols. Here we report the characterization and measurement of the polyphenol metabolome in urine samples from the EPIC cross-sectional study. Firstly, Phenol-Explorer, a web knowledge base on polyphenols, was interrogated to retrieve the identities of all known metabolites and gain an insight into their pharmacokinetics and biotransformations. Secondly, 24-hr urine samples from 481 subjects from the

EPIC cohort were subjected to non-targeted metabolomics profiling by high-resolution mass spectrometry to search for biomarkers of commonly-consumed polyphenol-rich foods. Thirdly, targeted metabolomics profiling of 38 different polyphenols was performed on the same EPIC samples using differential labelling with 13C-/12C-labelled dansyl chloride and LC-MS quantification. Phenol-Explorer details 303 metabolites identified in human or animal blood or urine and 501 polyphenolic food components. These data were utilized to screen metabolic profiles of 24-hr urine samples from the EPIC cross-sectional study. A total of 83 polyphenol metabolites varied in concentration within and between subjects from four European countries, reflecting variations in individual intake of polyphenol-rich foods. A robust method for quantification of 38 polyphenols was then developed. The use of 13C-labelled dansyl chloride to derivatize phenolic groups in all metabolites considerably increased the sensitivity and reliability of measurements. Polyphenol metabolites were detected in all or a large fraction of subjects according to their occurrence in foods and food preference, and their concentrations spanned five orders of magnitude. The most concentrated compounds in urine were found to be small phenolic acids that are known to be microbial breakdown products of more complex phenols. Polyphenols also clustered by overall dietary profiles across all samples. Urinary concentrations of some were correlated with reported intakes of known polyphenol-rich foods and could be used as biomarkers of food intake. The most comprehensive analysis so far of the polyphenol metabolome in cohort studies and its relationship with disease risk.

oral 120	15 min	food & health	William Edmands * Early Career Scientist

Mining the Human "Conjugatome" for Exposome-Wide Association Studies (EWAS).

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A major proportion of risk for non-communicable diseases (NCDs) in humans has been attributed to non-heritable components of cellular systems. Phase I and II conjugation reactions function to remove compounds of primarily exogenous origin. Levels of all conjugated chemicals in human biofluids (“conjugatome”) could be utilized to establish chemical exposures that may contribute to risk of NCDs. We demonstrate that sensitive liquid chromatography mass spectrometry (MS) instrumentation, a novel MS/MS acquisition approach and sophisticated bioinformatics tools (CompositeMS2Explorer) allow comprehensive characterization of the human conjugatome. Application of this approach to urine samples from the European Prospective Investigation into Cancer and Nutrition (EPIC) study revealed that the largest fraction of MS features detected (>60%) were conjugated metabolites characteristic of detoxification pathways. EPIC study urine samples (n=476) were profiled in negative mode electrospray ionization (ESI) using ultra-high performance liquid chromatography coupled to a quadrupole time-of-flight mass spectrometer. Online “iterative exclusion” data-dependent MS2 acquisition was conducted on a pooled quality control sample during the MS1 profiling acquisition. Correlation coefficients (Spearman's Rho) between dietary records and urinary compound levels were calculated in R. New software CompositeMS2Explorer was developed in R to match MS1 peak tables to MS2 fragmentation data. Metabolite substructure was interpreted by both customizable annotation from the literature and partitioned insilico fragmentation. Variable MS2 spectral noise was addressed by dynamic noise filtration and summed signal composite spectra generation. Substructure and pH dependent lipophilicity as orthogonal parameters for metabolite identification were also calculated. Using CompositeMS2Explorer we were able to match >1500 unique MS1 peaks to noise filtered composite MS2 fragmentation spectra. Of these >1200 were annotated with >250 substructures (~22% related to Phase II detoxification) from a database of literature-based characteristic neutral loss, product ions and electrospray adducts. This untargeted analysis revealed that more than 60% of the observable MS2 matched features corresponded to Phase II enzymatic detoxification transformations. Of the Phase II detoxification conjugates detected, the competing sulfate and glucuronide conjugation pathways in particular were highly represented (~43% and ~44% respectively) and in addition a significant number of glycine (~8%) and mercapturic acids (~5%) were also observed. More than 900 of the MS2 matched annotated features had significant pairwise-correlations (Spearman's Rho >0.6) and when combined with EPIC lifestyle and dietary co-variates it was revealed that a large proportion of these could also be attributed to dietary sources and common drugs. The principle function of enzymatic detoxification is to facilitate removal of xenobiotics, carcinogens and pro-carcinogens, primarily in the liver. Although various endogenous metabolites are also conjugated by Phase II biotransformation pathways, including hormone sulfation and fatty-acid-glycine conjugation, the vast majority of conjugated compounds originate from exogenous small molecules.Quantitative estimation of the many hundreds of

Phase II detoxification conjugates in MS1 metabolic profiles could also provide a surrogate of detoxification phenotype. For instance, epidemiological investigations have revealed enzymatic genetic polymorphisms in Phase II detoxification associated with many chronic diseases including many cancers. Thus, the sum of all detected detoxification conjugates could facilitate characterization of meaningful xenobiotic exposures arising from the diet, drugs and pollution sources as well as individual differences in fundamental metabolic pathways. The analytical and computational methods establish the human conjugatome as an important subset of the human exposome that has been suggested to constitute the “dark matter” of NCDs. A combined analytical and bioinformatic workflow provides insight into inter-individual exposures and detoxification phenotypes in free-living populations.

oral 121	15 min	food & health	Tomas Cajka

LC-SWATH-MS-based lipidomics for nutritional metabolic phenotyping in response to a dietary challenge containing gamma-linolenic acid

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Recent advances in liquid chromatography-mass spectrometry (LC-MS) have revolutionized metabolomics analysis by simplifying the analytical protocols and by increasing the chromatographic separation power and sensitivity of detection. For identification of metabolites based on MS/MS spectra, data dependent acquisitions (DDA) are competing with data independent acquisitions (DIA). DIA methods promise advantages in overall profiling coverage by not missing low abundant precursor ions. However, since the precursor ions for MS/MS spectra are not well-defined, deconvolution of MS/MS spectra and assignment of purified MS/MS spectra to MS1 precursor ions by computational methods is needed. For the extraction of plasma lipids, cold methanol and methyl tert-butyl ether were used. After evaporation of an aliquot and re-suspension, extracts were analyzed using a Waters UPLC CSH C18 column within a 15-min gradient. The QTOFMS instruments (AB Sciex TripleTOF 5600+) was operated in ESI(+) and ESI(–). Sequential windowed acquisition of all theoretical fragment ion mass spectra (SWATH) was performed using a 20-Da isolation window over the mass range m/z 300–1100. MS-DIAL software was used for automated mass spectral deconvolution of high-resolution MS data followed by MS/MS library search (LipidBlast). For quantification of identified lipids, 12 class-specific internal standards were used. Quality control samples were injected after every 10 actual samples. Using an optimized gradient for RPLC separation, information-rich chromatograms of plasma lipids were obtained with relatively narrow peaks (5–15 s) covering 12 lipid classes. To achieve a sufficient number of data points per chromatographic peak, a cycle time of 500 ms (2 data points/s) was set up consisting from 100 ms accumulation time for MS1 and 10 ms accumulation time for each 20-Da SWATH segment over the mass range m/z 300–1100. High-resolution MS data were processed using the novel open-source software, MS-DIAL. During MS/MS deconvolution, product spectra were first extracted for each precursor peak on all MS/MS chromatograms. In the next step, pure MS/MS spectra were determined from the peak heights of reconstructed chromatograms, thus removing background noise and deconvoluting pure spectra from co-eluting interfering metabolites. This methodology was applied on blood plasma samples collected from healthy participants before and after consumption of a dietary challenge containing gamma-linolenic acid (GLA) and a matched control. In a single blind, placebo-controlled, crossover design, seven healthy subjects received a dietary challenge in which the fat in the meal consisted of GLA or a control fat on 3 separate test days each. Plasma lipids were measured at 0, 2, 4, 6 and 8 h in response to the dietary challenges. On average plasma GLA was 17-fold higher in response to the GLA challenge compared with baseline and the control meal. Higher concentrations were also observed for minor lipids such as LPC(18:3), LPE(18:3), PC(16:0/18:3), PC(18:2/18:3), PC(18:3/18:3), PE(18:2/18:3), DG(18:2/18:3), DG(18:3/18:3) and acylcarnitine(18:3). Several subjects were identified as responders in elongating GLA into gamma-homo-linolenic acid from the dietary challenge containing GLA. However, the GLA challenge influenced mainly the pattern of blood plasma triacylglycerols (TGs). Out of the 103 identified TGs, 21 TGs containing GLA were detected with on average of 6-fold higher concentrations. A lipidomics workflow with comprehensive mass spectral fragmentation analysis (SWATH) and automated mass spectral deconvolution of high-resolution MS data.

oral 122	15 min	food & health	Raquel Cumeras * Early Career Scientist

Detection of fungal metabolites using non-invasive techniques from Gallus gallus domesticus eggs.

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The porosity of the outer poultry eggs shell allows pathogens to arrive inside egg by penetrating the eggshell. Under humid ambient storing conditions in refrigerators, eggs can be colonized by fungi and bacteria. Volatile organic compounds (VOCs) are produced by bacteria and fungi as they proliferate, and these chemicals are emitted back through the eggshell into the environment. Some of these compounds have low molecular weight and high volatility, and almost 200 VOCs have been previously identified from contaminated eggs. In this work, we present the study of metabolites from fungi inside hen eggs and their correlation with the fungi species-specific VOCs. Fresh-eggs(n=2) and 2months old-eggs(n=4) were stored under normal ambient humid conditions, and they were analyzed using non-invasive techniques: Solid Phase Microextraction Headspace Gas Chromatography Mass Spectrometry(SPME-HS-GC/MS) and Headspace Gas Chromatography Differential Mobility Spectrometry(HS-GC/DMS). Eggs were placed inside vials (neck-size-70mm,PTFE-cap,capacity-240ml) that were previously cleaned in a vacuum-oven(160ºC,3h), and samples were taken after flushing the vials with dryUHP air for 2min. Upon experimetns completion, all eggs were opened to check fungus presence and identification. DNA was extracted from mycelia of fungal cultures on APDA, and bacterial colonies that were grown on KB medium. PCR products were sequenced and the fungi and bacteria were identified using NCBI BLAST results. Petri dishes with grown fungi from eggs were analyzed with SPME-GC/MS for metabolite confirmation. Almost 100 VOCs have been identified with GC/MS and are classified in 5-groups: fungi (present only in the eggs containing fungi); old (present only in the old eggs that may or may not contain the fungi); healthy (present only in the eggs not containing the fungi); fresh (present only in the fresh eggs) and all eggs (present in all eggs). Specific compounds that have been previously attributed to the identified fungi (Botrytis cinerea and Cladosporium macrocarpum) have been matched with the identified compounds from the fungi-infected eggs as well as from the analyzed colonies that were grown in vitro in petri-dishes. GC/MS results allow for clear differentiation between non-infected eggs and eggs infected with fungi. Similarly to GC/MS results, approximately 100 VOCs that are present in the eggs and absent in the vials with dry air blanks have been identified in positive ion mode with GC/DMS. Additionally, 4 VOCs have been identified in the negative ion mode. The advantage of GC/DMS is that it can detect both positive and negative ions in a single measurement, while only one ion mode could be used at a time in some mass spectrometers . Although chemical identification with GC/DMS sensor may be not straightforward or not possible, the sensor can be useful as a VOC monitoring tool in production processes. Visual inspection of tested eggs revealed differences between non-infected eggs and eggs infected with fungi. Non-invasive sampling of eggs pathogens. GC/DMS chemical signatures to be potentially used in production process.

oral 123	15 min	food & health	Aoife O'Gorman * Early Career Scientist

Exploring the links between diet and health: A lipidomic approach.

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Epidemiological and clinical studies have shown clear evidence that a number of diseases with high rates of morbidity and mortality are associated with diet including diabetes, cardiovascular disease and cancers. Therefore, reliable dietary assessment methods are essential when attempting to understand the complex links between diet and health. Traditional tools for collecting dietary exposure are associated with errors, thus there is a growing interest in identifying biomarkers to provide a more accurate measurement. Metabolomics is a tool that offers huge promise in this area. The objective of this study was to use a multivariate statistical strategy to link lipidomic profiles with dietary data and biochemical health parameters to identify links between diet and health The relationship between lipidomic profiles, dietary data and biochemical parameters in volunteers (n=188) from the Metabolic Challenge Study (MECHE) was assessed. A food frequency questionnaire (FFQ) was used to assess dietary intake. Biochemical analysis was performed on the plasma samples using an RxDaytona analyser to measure glucose, cholesterol, TAGs, glucose, HDL, LDL. Lipidomic analysis was performed using high- throughput flow injection ESI-MS/MS (Biocrates Life Sciences AG). Principal component analysis (PCA) was applied to the lipidomic data set to reduce the data into principal components/lipid patterns (LPs). Linear regression and heatmap analysis were used to identify potential relationships between LPs, diet and health The lipidomic data set used for exploration had a total of 229 lipids from the following lipids species; acylcarnitines, lysophosphatidylcholines (LPCs), phosphatidylcholines (PCs), sphingomyelins (SMs), lysophosphatidylethanolamines (LPEs), phosphatidylethanolamines (PEs), phosphatidylserines (PSs) and ceramides (Cer). A total of 8 lipid patterns (LPs) were identified by PCA analysis, accounting for 65% of the total variance. These LPs were defined based on their major contributing lipid species. It was found that dietary fat intake had a significant relationship with LP1 and saturated fatty acid (SFA) intake had significant relationships with LP1, LP4 and LP8. LP1 was dominated by positive loadings for PEs, LP4 with PCs and LP8 had major positive loadings from the PC and PE lipid species, indicating that dietary fat intake has a relationship with these particular lipid species. Heatmap analysis was applied to visualise which specific lipids had strong positive correlations with fat intake. A preliminary panel of 13 lipids were identified as having strong relationships with dietary fat intake. These lipids were mainly PCs and acylcarnitines. Importantly, two of these lipids (PCaeC36:4 & C5) demonstrated a significant quantitative response to increased fat intake in a separate study. Further analysis of these lipids revealed significant relationships with health parameters. More specifically HOMA-IR had significant relationships with PCaaC40:4 & PCaeC36:4 and TAG had significant relationships with PCaaC40:4 & C5. Furthermore, preliminary results identified significant relationships between the inflammatory markers CRP and ApoB with C5 and PCaaC40:5 respectively, suggesting a link between fat intake and inflammation. Overall, the present approach has identified lipids that are related to dietary fat intake which in turn have significant relationships with inflammatory and insulin resistance markers. The present results pave the way forward for use of dietary biomarkers in health research. Identification of specific lipids that directly link dietary fat intake to health parameters.

oral 124	30 min	systems biology of cancer metabolism	Andrew Lane

Antagonistic effects of MYC and hypoxia in channeling glucose and glutamine into de novo nucleotide biosynthesis

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Cell proliferation requires up regulation of nucleotide biosynthesis over rate for both for making new DNA and RNA to support protein biosynthesis. MYC is a major transcription factor that regulates metabolic processes essential for cell division, and is overexpressed in many cancers. The nutrient sources and integration of the metabolic pathways for nucleotide biosynthesis that enable MYC-dependent cell division are poorly defined [1]. Using Stable Isotope Resolved Metabolomics (SIRM) [2] we have determined the fate of atoms from 13C6-glucose, 13C5,15N2-glutamine, or 2H-glycine into nucleotides under varied conditions of MYC expression in the MYC-inducible P493-6 B-lymphocyte [3] and several lung cell lines. P493-6 cells, A549, PC9 and HPLD1 cells were grown in the presence of [U-13C]-

glucose, [U-13C,15N]-Gln or [2H2]-Gly for one cell division. P493-6 cells, which have an inducible MYC gene were grown under four sets of conditions: MYC On/Off under 21% or 1% oxygen. Media samples were taken at timed intervals, the cells were harvested, and extracted immediately to provide polar and non-polar metabolites [4]. The resulting metabolites were analyzed by high resolution NMR and FT-ICR-MS to determine the isotopomer distributions in the free nucleotides and metabolites that characterize the related pathways of glycolysis, pentose phosphate shunt, serine pathways, Krebs cycle, glutathione synthesis and the purine and pyrimidine biosynthetic pathways. In parallel, gene expression data and MYC promoter occupancies were interrogated. MYC increased incorporation of 13C from glucose and glutamine into newly synthesized glycine and aspartate, which are channeled into nucleotides. MYC suppression in lung adenocarcinoma PC9 and A549 cells induced opposite effects on carbon flow into nucleotides. Exogenous 2H-Glycine was a relatively poor source for ATP synthesis but was preferentially incorporated into glutathione both in transformed cells and primary lung HPLD1 cells, whereas glucose-derived 13C Gly was incorporated into purines. Although hypoxia enhances glycolysis and maintains ribose synthesis in P493 cells, it antagonizes channeling toward nucleobase synthesis and reduces MYC-induced proliferation. MYC increased coordinately expression of the relevant metabolic genes associated directly and indirectly with nucleotide biosynthesis, which correlates with cell proliferation and cell cycle distribution data [3]. These results reveal the coupling of bioenergetics and nutrient availability to cell proliferation through regulation of metabolic channeling in de novo nucleotide biosynthesis. MYC coordinately regulates genes for nucleotide biosynthesis as well as the associated pathways for serine and glycine synthesis, which feed into the purine pathway. MYC up regulates the serine/glycine synthetic pathways for nucleotide MYC enhances channeling of glucose-derived Gly into purines and Gln-derived Asp into pyrimidines. Hypoxia diminishes glucose-driven Krebs cycle, and nucleobase but not MYC-induced ribose synthesis.

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oral 125	15 min	systems biology of cancer metabolism	Thomas Hankemeier

Studying individual differences in cancer drug efficacy using cell lines derived from patients

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Cancer drugs are known for their narrow therapeutic window and the need for understanding and predicting efficacy and toxicity. To understand the variation in cancer drug efficacy we studied the effect of a set of nine cancer drugs on more than 200 patient derived lymphoblastoid cell lines. Cytotoxicity was determined by the half maximal effective concentration (EC50). The aim of the project is to identify metabolites and pathways predicting sensitivity to cancer drugs. Metabolomics data were combined with baseline mRNA expression and GWAS data available for these cell lines. We determined the metabolic profile of the cell lines at baseline. For this we isolated the intracellular metabolites using an optimized protocol and analysed them subsequently with LC-MS/MS and targeted metabolomics. One of the target platforms covered a wide range of biogenic amines. Metabolites predicting EC50 of nine cancer drugs (docetaxel, doxorubicin, epirubicin, everolimus, gemcitabine, metformin, paclitaxel, rapamycin, arabinosylcytosine) were identified using univariate and multivariate statistics. For a small set of extremely sensitive and resistant cell lines, metabolomics profiles were measured before and after exposure to two anthracyclines,

doxorubicin and epirubicin. The metabolites predicting EC50 were correlated with mRNA expression at baseline, and with the genetics data. We first investigated the similarity of the variation in cytotoxicity and the metabolite profiles for the more than 200 cell lines. We found that from the nine drugs tested, the similarity in drug response and metabolite profiles was the largest for drugs from the same class. For example, doxorubicin and epirubicin, both anthracycline drugs, were rather similar in drug response and metabolite profiles. For both drugs, metabolites could be identified to predict sensitivity (and resistance) to the drug using the EC50 value of the cell line at baseline. Actually, some of the metabolites were significant for both drugs to predict the EC50 both such as for example 2-aminoadipic acid. Next, we selected a few cell lines that were extremely sensitive or resistant to doxorubicin and epirubicin. For these we acquired the metabolic profile before and after drug exposure. Using the metabolic change due to drug treatment we could identify metabolites to differentiate between sensitive and resistant cell lines for doxorubicin and epirubicin. Some of the metabolites were in common for both drugs such as taurine and 2-aminoadipic acid, and some of the metabolites were different. This actually reflects the to a large extent common mechanism of both drugs. Finally, we correlated the metabolites predicting drug response to mRNA at baseline (i.e. without drug exposure) and GWAS to support the interpretation of the results. Metabolomics on a wide panel of patient derived cell lines can reveal insights in individual variation of cancer drug response

oral 126	15 min	systems biology of cancer metabolism	Ulrike Rennefahrt

Metabolite profiling reveals the glutathione biosynthetic pathway as a therapeutic target in triple negative breast cancers

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Identifying metabolic pathway alterations that are critical to support cancer growth is a key hurdle for developing therapeutic strategies that exploit these pathways. Triple-negative breast cancer (TNBC) is an aggressive form of breast cancer that represents about 15-20% of all breast cancers. Because TNBC tumors do not express the estrogen or progesterone receptor and lack HER2 amplification, the disease is not responsive to current targeted therapies. The development of therapeutic approaches specific for TNBC is hindered by genetic heterogeneity, the lack of common oncogenic drivers, and significant efforts are being made to subtype the disease. We have applied metabolomic and pharmacological approaches to subtype the metabolic state of TNBC cancer cells and to unravel targetable pathways in TNBC. For the identification of dysregulated metabolic pathways in TNBC, we conducted mass spectrometry-based metabolomics of 12 TNBC cell lines representing all six TNBC subtypes previously defined by gene expression (Lehmann et al. J Clin Invest. 2011) and control cells (primary and non-transformed breast epithelial cells). A robust, untargeted broad-spectrum mass spectrometry-based platform including LC-MS/MS and GC-MS was used to semi-quantitatively measure a wide range of metabolites in cell lysates. The relative levels of 155 intracellular metabolites distinguished TNBC from non-transformed breast epithelia, and revealed two metabolic subtypes within TNBC that, unexpectedly, correlate with markers of basal-like versus non-basal-like status. Distinguishing metabolites included amino acids, lipids and the cellular redox buffer glutathione. Levels of glutathione were generally lower in TNBC cell lines compared to controls, and markedly lower in the metabolic subtype containing non-basal-like TNBC. Further, these cell lines showed enhanced sensitivity to inhibition of glutathione biosynthesis, demonstrating a dependence on glutathione production for survival. These findings demonstrate the potential of targeting the glutathione biosynthetic pathway as a therapeutic strategy in TNBC, and suggest that existing clinical biomarkers may provide a means for stratifying TNBC tumors to identify likely responders to anti-glutathione therapy. (1) Targeting glutathione biosynthesis as therapeutic strategy in TNBC. (2) Existing clinical biomarkers may provide a means for stratifying TNBC.

oral 127	15 min	systems biology of cancer metabolism	Tokuwa Kanno * PhD student

Integration of 1D-HRMAS-NMR and Transcriptome Data Reveals Novel Therapeutic Targets of Rho/ROCk Driven Melanoma Metastasis

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90% of cancer related deaths are caused by metastasis, the process by which cancer cells spread throughout the body. Altered cell migration and invasion are regulated by actomyosin contractility and underlie the metastatic cascade. Rho GTPases and their downstream targets Rho Kinases (ROCK) play a central role in increasing actomyosin contractility. Highly invasive cells found at metastatic sites and the invasive front of tumours have an amoeboid morphology characterised by high acto-myosin contractility. The purpose of our study is to identify novel therapeutic targets regulating cancer progression and metastasis by integration of metabolomic and transcriptomic data. We have validated some targets in vitro by analysing morphological features and proliferation after RNAi knockdown of these candidate targets. ROCK signalling was altered in human and mouse melanoma cells using: a) ROCK small molecule inhibitors (SMI) b) ROCK1 and ROCK2 double siRNA c) ROCK1, ROCK2 and double ROCK1/2 Knock-out models of melanoma. In the third instance ROCK depletion was achieved by crossing ROCK1 and ROCK2 conditional knock-out mice with a mouse melanoma model driven by BRaf V600E (Tyr-Cre-ERT2). ROCK1 and ROCK2 alleles that were sandwiched by the loxP sites were deleted upon treatment with tamoxifen which drives expression of Cre-recombinase. Samples were studied using ^{1}H High Resolution Magic Angle Spinning NMR. Principal Component Analysis and Orthogonal Partial Least Squares Discriminant Analysis with cross validation were used to identify metabolites correlated with Rho-ROCK signalling. Candidate genes from altered metabolic pathways were identified by analysing microarray data from A375M2 human melanoma cells treated with ROCK SMIs and patient data taken from six public databases. Gene expression and survival analysis were analysed. Candidate genes were targeted by siRNA in A375M2 cells and the effects on cell morphology and actomyosin contractility were studied using confocal microscopy and western blotting. Altering ROCK activity in all studied systems showed significant effects on cellular metabolism. The three different ROCK inhibitors showed very similar metabolic effects on choline, amino-acids, and myo-inositol intracellular concentrations. siRNA knockdown of ROCK in the same cell lines identifies which metabolic effects are specific to ROCK activity and which are due to off-target effects of the small molecule inhibitors. Samples from knockout mice showed changes consistent with previous data; in addition they revealed potential independent function of the two ROCK isoforms, ROCK1 and ROCK2 which appear to be additive in the double Knockout. After Metabolomic data was integrated with microarray data and analysed with metaboanalyst 3.0, we decided to further investigate glutamine/glutamate metabolism in malignant melanoma. Genes involved in glutamine/glutamate metabolism were analysed in transcriptome databases. The criteria for inclusion in the candidate library were a) that gene expression was increased 1.5x at $p<0.05$ when comparing metastatic samples to primary tumour samples, and b) that genes needed to pass these criteria in at least three of the databases analysed. 20 candidate targets were selected for analysis by RNAi screen. The RNAi screen is ongoing, however certain candidate genes already show promise in altering actomyosin contractility to a similar level of Rho-ROCK. Biochemical interactions between ROCK and candidate genes are also being investigated. These preliminary results demonstrate the potential of integrating -omics data to reveal currently overlooked therapeutic targets in metastasis. In addition, our studies have revealed novel downstream targets of the Rho/ROCK signalling pathway. Integrated metabolomic, transcriptomic and patient survival data reveal how Rho/ROCK signalling regulates cellular metabolism for novel therapeutic targets in metastasis.

oral 128	15 min	systems biology of cancer metabolism	Luisa Doria * PhD Student

A systems biology approach for the classification of ovarian cancer specimens by desorption electrospray ionization mass spectrometry

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Ovarian cancer is the most lethal female reproductive cancer. The “gold standard‟ for diagnostics is tissue biopsy and histopathological assessment. There is an increasing demand for ovarian cancer diagnostics with improved safety, efficiency and lower costs. Desorption Electrospray Ionization-Mass Spectrometry Imaging (DESI–MSI) has the potential to deliver systems oriented cancer diagnostics augmenting cellular morphological information on cellular metabolic content. The approach also represents an opportunity to investigate tumour biology from an entirely new perspective. Lipids represent an important class of metabolites with unique, however poorly understood role in cancer physiology. This study applied a “top-down” systems biology oriented approach for cancer diagnostics by examining the concentration changes of hundreds of lipid species in epithelial ovarian carcinoma tissues by DESI-MSI. 50 ovarian serous adenocarcinoma, 10 epithelial ovarian carcinoma (mucinous and endometroid) and 10 healthy ovarian tissue samples were collected, cryosectioned and analysed by DESI-MS in positive and negative ion mode. The sample slides analysed by DESI were also stained by haematoxylin and eosin (H&E), digitalised and examined by histopathology professionals, in order to align the optical and MSI image for precise selection of region of interests (ROI). The acquired DESI-MSI data were pre-processed to account for common bio-analytical complexities by median fold change profile normalization and log-based variance stabilizing transformation. The processed MSI data were subjected to supervised multivariate statistical analysis using Recursive Maximum Margin Criterion (RMMC) discriminant analysis to identify patterns of diagnostic and prognostic importance. Firstly, MSI pixels obtained from each different tissue types (tumour, tumour surrounding stroma and healthy (“control”) stroma) were annotated with the use of H&E stained images by qualified histopathologist. For each sample, supervised multivariate data analysis was performed using RMMC. Leave region out cross-validation results exceeded 98% accuracy for both negative and positive ion mode for classification of these areas within individual tissue samples. Multivariate statistical models were also created using the entire dataset of samples together. Supervised MMC analysis clearly differentiated all tissue types with a leave patient out cross-validation performance of ~80% for both ion modes. Lipid composition was analysed in more depth based on a possibility of significant alteration of lipidomic profile in cancer vs tumour associated stroma and healthy tissue. Potential lipidomic signatures discriminating cancer from associated stroma and control (healthy stroma) were analysed using analysis of variance tests adjusted by means of Hochberg–Yekutieli procedure to control false discovery rate at 5%. Interestingly, the lipid classes having the highest contribution to the classification of cancer tissue were phosphatidylethanolamines, followed by the phosphatidylserines in negative ion mode. In case of positive ion mode data, despite the phosphatidylcholine class being the most abundant, phosphatidylethanolamines again were the most significant to separate the two tissue types. Phosphatidylethanolamines, present in the inner part of the membrane, play a critically important role in the initial steps of signalling pathways, cell governing and proliferation. The analytical results were associated with clinical metadata and significant correlations were found between clinical phenotype and the lipidomic profile of cancer tissues. In conclusion, it was possible to analyse cancer tissue in two different and very promising ways: the generation of metabolic profiles by DESI-MS to better understand the lipid metabolism in cancer progression, and its distribution to assist with diagnosis and prognosis at histological level. Imaging of cancer tissue using DESI-MS generates lipidomic profiles and distribution, which can augment histological diagnosis of epithelial ovarian carcinoma.

oral 129	50 min	special invited session	John Ryals

Metabolomic technology advancements breathe life into personalized medicine

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Genomics has been a focal-point for personalized medicine throughout the last decade. And, while genomics has provided many important insights and discoveries a staggeringly complex picture has also emerged. High allelic variation, the polygenicity of most traits of interest, a majority of mutations of interest residing in non-coding regions, and elusive influences like the microbiome and epigenetics are just a few added complexities that have been illuminated since the genome was first sequenced. In response to this complexity, many investigators are using a variety of orthogonal data types to augment genomics (e.g. proteomics). All of these data types aim to derive an "intermediate phenotype". Since metabolites are regarded as an intermediate phenotype, metabolomics is viewed as a potential solution. But, in order for metabolomics to be effective for profiling large clinical cohorts, platforms must be highly stable, scalable and able to capture a meaningfully broad swath of the metabolome. Perhaps most important to deriving meaningful associations of metabolites to genes (particularly to rare alleles) is that the data must have exceptional precision and accuracy. In this lecture, the key technological innovations that have fulfilled the above criteria, and therefore poise metabolomics, as a first-line tool for personalized medicine will be described. In parallel to these innovations recent research on large populations show how metabolomics is revealing important information about the phenotypic and genetic basis of the phenotype. In closing, it will be illustrated how the findings from large population studies, coupled to the technology advancements, have opened-up new avenues for personalize health and disease assessment (so-called n-of-1 studies) from inborn error testing to assessment of individual risk and response. The summation of the maturity of the technology, these recent research results, and the needs in the genomics community indicate a strong presence for metabolomics in the continued pursuit of personalized medicine.

POSTERS

POSTER 001

Evaluation of neoadjuvant chemotherapy using MR metabolomics

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Neoadjuvant chemotherapy is used in the treatment of locally advanced breast cancer to make it operable or to enable breast conserving surgery. Bevacizumab is an antiangiogenic agent which has been found to improve progression free-survival when combined with chemotherapy for initial treatment of metastatic breast cancer. Here, we investigated the metabolic effects of neoadjuvant chemotherapy with and without bevacizumab in non-metastatic breast cancer using magnetic resonance spectroscopy (MRS). The study had a longitudinal design allowing for multilevel approaches to investigate metabolic differences between patient groups. Identification of these differences may allow for assessment of patient benefit to treatment and the understanding of underlying biological mechanisms affecting response. The metabolic profiles of 122 breast cancer patients (n=270 tumor tissue samples) were determined by high resolution magic angle spinning MRS. All patients received FEC and taxanes as chemotherapeutic neoadjuvant treatment, while they were randomized to receive bevacizumab or not (controls). Biopsies were sampled prior to treatment (T1), after12 weeks (T2), and at surgery (T3). PLS-DA, validated by permutation testing, was used to build classification models at each time point to discriminate responders from nonresponders, bevacizumab-receiving patients from controls, and bevacizumab-receiving responders from control responders. Multilevel-PLSDA was applied to spectra paired by time point (T1 and T2, T2 and T3, T1 and T3). Relative levels of 16 metabolites were calculated by integration and analyzed by univariate linear mixed models (LMM). Principle component analysis showed clear changes in metabolic profiles as an effect of chemotherapy. PLS-DA revealed metabolic differences between responders and nonresponders at T3, but not T1 or T2, with an accuracy of 77% ($p<0.001$), with responders showing higher glucose and lower glycerophosphocholine, phosphocholine, choline and glycine levels. Bevacizumab-receiving patients and controls, and bevacizumab-receiving responders and control responders could not be discriminated at any time point. Multilevel-PLSDA (paired multivariate analysis) showed significant separation ($p<0.05$) of earlier time points from later time points in all cases, confirming a metabolic effect due to treatment as observed in PCA. However, loading plots were similar when comparing responders from nonresponders, bevacizumab-receiving patients and controls, and bevacizumab-receiving responders and control responders. Thus, no metabolic differences could be detected between these subgroups. Univariate unpaired LMM revealed significant differences in 13/16 metabolites for the factor “time” after FDR correction, while only lactate was significant for “response” and none for “bevacizumab”. Glucose has been found to be lower in cancer, while lactate is higher due to increased anaerobic glycolysis or the Warburg effect. The choline containing metabolites have been found to be elevated in tumor tissue when compared to normal, and are involved in the metabolism of phosphatidylcholine, the most abundant phospholipid in eukaryote cell membranes. Glycine level has been correlated with proliferation rate and axillary lymph node spread. Thus, the metabolic findings with respect to chemotherapy and response are in agreement with tumor reduction. We will further investigate possible mechanisms through which treatment leads to different outcomes in response according to the metabolic differences found. Metabolic profiles were able to discriminate responders from nonresponders after neoadjuvant chemotherapeutic treatment of non-metastatic breast cancer.

POSTER 002

The Role of Mutations that Impair Iron Sulfur Cluster Delivery in SDHB-Deficient Cancer Syndromes

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Background: Mutations in the Fe-S cluster-containing SDHB subunit of succinate dehydrogenase cause familial cancer syndromes. Recently the tripeptide motif L(I)YR was identified in the Fe-S recipient protein SDHB, to which the co-chaperone HSC20 binds, together with the Fe-S cluster scaffold, ISCU, and the chaperone, HSPA9. In order to characterize the metabolic basis of SDH-deficient cancers we utilized stable isotope-resolved metabolomics to characterize a novel SDHB-deficient renal cell carcinoma cell line and performed bioinformatics and biochemical screening to analyze Fe-S cluster acquisition and assembly of SDH in the presence of other cancer-causing SDHB mutations. We found that the SDHB>R46Q</sup> mutation in UOK269 cells disrupted binding of HSC20, causing rapid degradation of SDHB. In the absence of SDHB, respiration was undetectable in UOK269 cells, succinate accumulated, HIF1a increased markedly, and glutamine became the main source of TCA cycle metabolites through reductive carboxylation. Biochemical and bioinformatic screening revealed that 37% of disease-causing missense mutations in SDHB were located in either the L(I)YR Fe-S transfer motifs or in the eleven Fe-S cluster-ligating cysteines. Conceptual framework why particular mutations disproportionately cause loss of SDH activity, accumulation of succinate and metabolic remodeling in SDHB cancers.

POSTER 003

Metabolic Flux Reprogramming of MCF7 Cells under Nutrient Restricting Conditions

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The metabolism of fundamental nutrients varies among cancer and healthy cells and this is being investigated as an important target in cancer therapy. Despite glutamine is the second most important substrate for cancer cells after glucose, the mechanisms of glutamine dependence and the response and resistance of cancer cells to glutamine deprivation have not been completely revealed. Analysis of the metabolic flux profile of cancer cells can be used to comprehend the regulation of cancer metabolism and point out new therapeutic strategies. In this perspective, the analysis of the isotopologue distribution resulting from incubation with stable isotope tracers using the software Isodyn, has been used to assess the metabolic flux profile of human breast adenocarcinoma cells MCF7 under glutamine deprivation. 13C assisted metabolic flux analysis (13C-MFA) has been used for flux determination. In short, MCF7 cells were incubated with [1,2-13C]-glucose at their exponential growth phase for defined times. Cells were counted before and after the incubations. Consumption and production of glucose, lactate and amino acids from incubation media were measured by spectrophotometry and High Pressure Liquid Chromatography (HPLC). The isotopologue distributions of several metabolites including TCA cycle intermediates were measured by Gas Chromatography-Mass Spectrometry (GC-MS). Information derived from the isotopologue distributions and biochemical measurements have been processed by our in-house developed software Isodyn in order to obtain the metabolic flux profiling of the MCF7 cells with deprivation of glutamine. Spectrophotometric measurements and mass isotopomer distribution analysis (MIDA) have revealed that glutamine deprivation leads to a metabolic reprogramming on various pathways including glycolysis, pentose phosphate pathway, TCA cycle and amino acid

incorporations. More quantitative and specific results derived by metabolic flux profile obtained by Isodyn also concords with the changes observed in these pathways. In that way, significant changes on the fluxes of certain key enzymes of the central carbon metabolism under glutamine deprivation have revealed promising targets on cancer treatment. The study now is being carried on with specific inhibitions of the specified pathways in order to further validate the effects of absence of glutamine and uncover the mechanisms lying behind. The analysis of the metabolic reprogramming that MCF7 cells undergo under glutamine deprivation could point novel targets in anticancer therapy.

POSTER 004

Metabolites as anticancer agents: the selective and interrelated toxicity of menaquinone and phosphoethanolamine

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The well-known potential for metabolites to exert regulatory or signaling control, coupled with the increasingly widely accepted importance of metabolism in the progression of cancer, suggests that metabolites could be viable leads for developing novel cancer therapeutics. Our previous work showed that metabolites predicted by a computational model to be depleted in cancerous cells relative to healthy cells often had a selective antiproliferative effect against the cancer cells when they were added as supplements to growth media. Here, we characterized the metabolic dynamics and other mechanisms at play in the anticancer activity of one of the strongest leads from our previous work, menaquinone (Vitamin K2), and from this identified another metabolite with selective anticancer activity, phosphoethanolamine. The Jurkat cell line and a noncancerous lymphoblast cell line were used as a model system to study the effects of menaquinone on acute lymphoblastic leukemia (ALL) cells, based on predictions from a previous computational model. Cells were treated with menaquinone, two chemotherapeutic positive controls, and a vehicle control. Two-dimensional gas chromatography coupled to mass spectrometry (GCxGC-MS) was used to measure intracellular metabolite profiles daily for all experiments. Characterization of the apoptotic effects of menaquinone, control chemotherapeutics, and the newly-uncovered anticancer activity of phosphoethanolamine was performed via flow cytometry using live/dead staining and multiple established markers for apoptosis. Menaquinone was found to have selective antiproliferative and toxic activity against Jurkat cells relative to non-cancerous lymphoblast cells, as previous experiments had suggested. In fact, at mild doses of menaquinone, the lymphoblasts actually experienced modest (though insignificant) growth stimulation over the first one to two days of treatment. GCxGC-MS-based metabolic characterization of the cells' response to menaquinone showed that non-cancerous lymphoblasts experienced very little metabolic change during treatment with menaquinone. In contrast, the menaquinone-treated Jurkat cells exhibited significant metabolic changes by 72 hours of treatment. Using principal component analysis, it was evident that these changes were fundamentally different than the metabolic impacts of other chemotherapeutics, which were included as a control for general cell death-related metabolic changes. Digging deeper, we identified a metabolite (phosphoethanolamine) whose levels in treated Jurkat cells increased even before substantial antiproliferative effects were observed, but whose levels did not change in the untreated Jurkat cells, chemotherapeutic-treated Jurkat cells, or any of the control lymphoblast experiments. This strong, unique effect suggested that phosphoethanolamine may actually be playing a functional role in the antiproliferative activity, perhaps by mediating the induction of apoptosis. To test this hypothesis we supplemented Jurkat and lymphoblast cells with phosphoethanolamine, and we found a selective antiproliferative effect of phosphoethanolamine on Jurkat cells relative to non-cancerous lymphoblast cells. We then characterized the mechanisms of antiproliferative activity, identifying that both molecules induce apoptosis and do so via similar pathway mechanisms. This work supports the potential for metabolites as anticancer agents or as therapeutic leads, and indicates the power of metabolomics to begin unraveling the mechanisms underlying the regulatory or signaling roles of these compounds. The cross-talk of metabolism and apoptosis is a promising field; we used metabolomics to explore metabolites' potential as therapeutic leads.

POSTER 006

Triple negative breast cancer biomarker identification for drug development

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To date, no targeted clinical therapies are available to treat triple negative breast cancer (TNBC). This highly aggressive form of breast cancer (BCa) derives its name from the absence of estrogen receptor (ER+), progesterone receptor (PR+), and HER2/neu receptor (HER2+). These receptors are expressed in other subtypes of BCa (i.e. Luminal, HER2-enriched) and have been successfully targeted with therapeutics such as Tamoxifen and Herceptin. New therapeutics are needed to improve the prognosis for patients with TNBC. We sought to determine the differences in metabolic pathways between TNBC and hormone responsive BCa cell types prior to and following chemotherapeutic treatment. Cell lines were grown in DMEM media with and without U-13C-glucose or U-13C- glutamine enrichment. Broad spectrum metabolomics and metabolic flux analysis (MFA), using NMR, were conducted on cell extracts to reveal differences in TNBC cell lines (MDA-MB-231 and MDA-MB-468) compared with hormone receptor-positive cell lines [(ER+ and +/-HER2+) (BT474 and MCF-7)] prior to and following treatment with the chemotherapeutic Taxol®/Paclitaxel. Inflammatory responses were also evaluated by cytokine profiling of the media. Identified marker metabolites and cytokines were integrated to determine key biological pathways using GeneGo (MetaCore) software. Broad spectrum metabolomics analysis revealed differences in the metabolic profiles between the TNBC and hormone-responsive cell lines in the absence of treatment, with a higher level of amino acids (e.g. alanine, glutamate, glutamine), tripeptide (glutathione), short chain fatty acids and ketone bodies (isobutyrate, β-hydroxybutyrate), nucleotides and nucleotide sugars (ADP, ATP, UDPOSTER glucose, UDPOSTER glucuronate) in the TNBC cell lines. Interestingly, an established breast cancer biomarker, choline and its metabolites were elevated in the hormone-responsive cell lines. MFA demonstrated that glucose utilization was greater in the TNBC cells (MDA-MB-468) compared to the Luminal A cells (MCF-7), as the TNBC cells derived glycine and glutathione (γ-glu-cys-13C-gly) from the media 13C-glucose, while the Luminal A cells did not. In response to Taxol treatment, more metabolites were altered in the hormone-responsive cell lines compared with the TNBC cells, indicating their increased sensitivity. Furthermore, a dramatic decrease in glutamine utilization in the Luminal A MCF-7 cell line was seen after Taxol treatment. Cytokine arrays were used to evaluate the secretory profile of 81 inflammatory cytokines revealing different treatment responses across the four cell lines. Most notably, there was a trend in more significantly downregulated cytokines in the least aggressive Luminal line, BT474 (ER+, PR+, HER2+) to more significantly upregulated cytokines in the most aggressive TNBC MDA-MB-468 cell line. Additionally, only significant downregulation of osteoprotegerin was seen in all four cell lines after treatment with Taxol. Integration of the differentiating metabolites and cytokines using GeneGo pathway mapping analysis identified Immune response_T cell subsets: secreted signals as the most perturbed pathway in both MCF-7 and MDA-MB-468 cells in response to treatment. Other significantly different altered biological pathways based on MCF-7 responses included Immune response_IL-17 signaling pathways and for MDA-MB-468 responses was Immune response_IL-4 – antiapoptotic action. This work can potentially determine which of these metabolites might serve as the best TNBC biomarkers and new therapeutic targets.

POSTER 007

Investigation of Metabolomic Blood Biomarkers for Detection of Lung Adenocarcinoma

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Lung cancer is the leading cause of cancer deaths worldwide. Of the major forms of lung cancer, non-small cell lung cancer (NSCLC) adenocarcinoma is the most prevalent histological type. Although low-dose computed tomography (LDCT) was recently shown to reduce mortality in high-risk patients, it is limited by high false-positive rates, costs, and potential harms. The identification of a complementary biomarker panel to enrich the population for whom a LDCT would be the most beneficial is needed. An untargeted metabolomics approach was utilized in a case-control study design of lung adenocarcinoma (ADC) to identify metabolomic signature differences in serum and plasma and then used to construct classifiers for diagnosis. Plasma and serum were collected from heavy smokers (current and former) with or without NSCLC ADC and used for two case-control studies (ADC1 and ADC2). There were 52 ADC cases (Stage I-IV) and 31 control in ADC1 and 43 ADC cases (Stage I-IV) and 43 controls in ADC2. Control and cancer cases were frequency matched for gender, age and smoking history. Plasma and serum were derivatized and then analyzed using gas chromatography time-of-flight mass spectrometry (GCTOF-MS). Differential analysis was performed and the top significant metabolites (FDR < 0.05) for each blood matrix were identified and selected for development of classifiers, singly or in combination, as a diagnosis means. The developed classifiers were validated in an independent test set (ADC2). Aspartate was determined to have the best accuracy (82.6%) and specificity (97.7%) for a single metabolite in serum whereas pyrophosphate had the best accuracy (75.6%) and specificity (90.7%) in plasma when tested in the test set. The multiplex classifier of four serum metabolites performed best with accuracy of 74.42%, specificity of 79.07% and sensitivity of 69.77% when tested in the test set. For plasma, a multi-metabolite classifier consisting of 9 metabolites gave an accuracy of 73.26%, specificity of 93.02% and sensitivity of 53.49% when tested in the test set. When comparing plasma and serum metabolome, 3 metabolites (maltotriose, glutamate and Bin_223618) were found to be consistently abundant in adenocarcinoma compared to controls. Comparison of overall diagnostic performance between the two blood matrices indicated that serum classifiers, both singly and in combination, provided slightly better diagnostic power. This study shows the great potential of metabolite-based diagnostic tests for early detection of lung adenocarcinoma. Further validation is warranted.

POSTER 008

Metabolite changes associated with methionine stress sensitivity of cancer

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Altered cellular metabolism has gained larger recognition as a hallmark of cancer in recent years. While mechanistic insights into the metabolic changes in cancer are limited, the importance of methionine metabolism in cancer cell proliferation has been known and studied for over 30 years. These early studies describe a “methionine-dependence” or “methionine stress sensitivity” of cancer as a phenomenon where the majority of cancer cells cannot proliferate in growth media in which methionine is replaced by its metabolic precursor homocysteine. Interestingly, non-transformed cells are unaffected by this metabolite replacement. Previously, we identified a cell cycle arrest in cancer cells as a response to methionine stress and a global metabolic shift toward a more oxidative, or quiescent, cell state. Using the breast cancer cell line MDA-MB468, we investigate the metabolic response to methionine stress by mass spectroscopy. As a control, we have derived rare clones from MDA-MB468 cells (referred to as MB468res) that are methionine stress insensitive and have lost their tumorigenic ability for anchorage independent growth. The MB468 and MB468res cell line pair is an ideal model to identify unique metabolic signatures linking methionine dependence and tumorigenicity. To analyze metabolite changes in both cell lines, we employed an untargeted approach and lipid analysis during methionine stress over a 48 hour time period. In order to assess homocysteine flux and related metabolites, cells were cultured with a deuterium labeled homocysteine molecule over a12 hour time period. Untargeted profiling of

primary metabolites revealed a significant response to media stress within two hours of the media shift, including metabolites involved in methionine metabolism and related pathways. Interestingly, the majority of metabolite abundances decrease, while S-adenosylmethionine (SAM), the down-stream metabolite of methionine and the major methyl donor of the cell, increases over time. This data suggest a possible global inhibition of SAM use during methionine stress conditions. Lipidomics analysis identified an inverse response to methionine stress by phosphatidylcholine (PC) and phosphotidylethanolamine (PE), in MB468 but not MB468res cells. In MB468 cells, PE levels steadily increase over the 48 hour time course whereas PC, a product of SAM and PE, declines. In contrast, levels of PC and PE increase during methionine stress in MB468res cells. Both PC and PE are prominent phospholipid species in eukaryotic membranes and decreased levels may reflect a cellular defect in using SAM as a methyl donor for lipid biogenesis. Our analysis of homocysteine flux suggest that the majority of homocysteine metabolism in both MB468 and MB468res cells is redirected towards the transsulfuration pathway for glutathione synthesis instead of the remethylation pathway. Although the remethylation pathway is the major source for methionine regeneration, efforts to prevent damage from reactive oxygen species by glutathione may be become priority during methionine stress. Previous studies using fluorescence lifetime imaging microscopy, suggest MB468res cells are able to tolerate a metabolic transition to oxidative phosphorylation unlike MB468 cells. This metabolic transition and response during methionine stress may play a large role in the methionine stress sensitivity of cancer. This study investigates metabolic vulnerabilities of cancer from an unexplored area of methionine metabolism.

POSTER 009

Multi-omics and in silico approach to decipher metabolic network shifts during the differentiation of a human hepatic cell line

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Many man-made chemicals present as contaminants in food and/or water are strongly suspected to induce adverse metabolic effects in Human. Liver is the key organ for xenobiotics biotransformation, and the use of metabolically competent cell lines is essential to explore the mechanisms underlying the metabolic effects of these substances. In this study, we combined multi-omics data and in silico methods to better characterize the metabolic capacities of HepaRG, a hepatic cell line increasingly used in toxicity studies. We exploited transcriptomic and metabolomic data in the context of the global human genome-scale metabolic network Recon2, which gathers the metabolic reactions the organism can perform and their associated genes, to compute a relevant sub-network, more specifically representing the functional hepatic metabolic network. Gene expression and metabolomic (NMR and LC-HRMS) data were obtained on the human hepatoma cell line, HepaRG. In a first step, we applied the iMAT algorithm developed by Shlomi et al. to identify, based on gene expression data, the sub-networks of reactions specifically active in HepaRG cells. The goal was to find a coherent model that maximizes the number of reactions whose activities match the expression state of the associated genes. Metabolomic data were then used to further select particular sub-networks. Metabolites identified by NMR or annotated from LC-HRMS were compared with metabolites predicted to be “present” in the models. Models displaying the higher adequacy with metabolomic data were selected. Using this method, we were able to predict which reactions of the global human metabolic network were active or inactive (activity state) in each cell stage.

80% of our predictions were consistent with the experimental transcriptomic and metabolomic data. Interestingly, almost 50% of the predicted activity states of reactions could not directly be inferred from transcriptomic data and were « newly » inferred from the computational model. Indeed, although gene expression data were available for about 25% of the reactions, the method allowed to predict the activity state of about 80% of the reactions, demonstrating that additional and novel information about cell-specific activity states of reactions is gained by using the stoichiometry and topology of the metabolic network, combined with metabolomic data. Plausible models selected after the inclusion of the metabolomic data did not necessarily display the best adequacy with the initial transcriptomic data, allowing us to predict that some genes were very likely either post-transcriptionally or post-translationally modulated. The method was applied to data obtained at two differentiation stages, namely 4-days culture (progenitor) cells and 30-days culture (fully differentiated) hepatocyte-like cells. For each stage, the predicted set of active reactions was found to constitute a model reflecting the specific functional metabolic network of HepaRG cells for the corresponding specific developmental stage. Comparison of predicted active reactions in these two metabolic network models provides key information about the metabolic changes occurring during the transition between these two stages. Many reactions predicted to be activated during the differentiation process were consistently found to belong to pathways specific to the development of the hepatic activity (bile acid synthesis, amino acid metabolism…) and anabolic/catabolic processes including detoxification functions (cytochrome metabolism). Such context-specific functional reconstruction is a first step in the determination of the metabolic capacities of key cell lines used in toxicology. Combining in silico methods with omics data enables to characterize global shifts in the developing hepatic metabolic network.

POSTER 010

Pairwise metabolite-metabolite correlation analysis (MMCA) of HR-MAS 1H NMR spectra from human brain tumours

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Detection of the percentage of necrosis in human brain tumours is important for estimating grade of malignancy and response to therapy. Human brain tumour tissues from glioblastoma multiforme (GBM), meningioma, oligodendroglioma and metastatic tumours were analysed by metabolite-metabolite correlation analysis of HRMAS 1H NMR spectra from the eTumour database. The following metabolites were quantified using a modified LC-Model basis set: alanine, choline, creatine, lactate, glutamine, glutamate, NAA, phosphocholine, taurine, myo-inositol and lipids/macromolecules.The estimated metabolite concentrations from LCModel fittings were used in the investigation of pairwise metabolite-metabolite correlations. In GBM samples negative correlations were observed between the small-molecule metabolites and lipids/macromolecules. Pairwise metabolite-metabolite correlations can serve as an overview of metabolism and can be helpful in understanding the cellular metabolism. All the HRMAS 1H NMR spectral data (water pre-saturated) were downloaded from the e-Tumour database (http://solaria.uab.es:9091/eTumour/). The eTumour project (2004 – 2009), funded by the EU (FP6-2002-LIFESCIHEALTH 503094), involved 21 partners across Europe and Argentina. Spectra of tumour tissue samples from verified cases of glioblastoma multiforme (GBM, n=59), oligodendroglioma (ODG, n=16), brain metastasis (n=13) and meningioma (n=9) were analysed in this study. LCModel was used with a modified basis set to estimate the metabolite concentrations from water-suppressed spectra; alanine, choline, creatine, lactate, glutamine, glutamate, NAA, phosphocholine, taurine, myo-inositol and lipids/macromolecules were quantifiable. The pairwise metabolite-metabolite correlations were estimated by using a method we recently developed; it treats as significant only correlations with $P \leq 0.001$. Only the GBM samples showed substantial numbers of pairwise correlations, perhaps because of the lower number of ODG, meningioma and metastasis cases in the eTumour database. Positive correlations are evident between many of the metabolites and also among the various lipid and macromolecule peaks (note, however, that the latter peaks mainly arise from functional groups, e.g. CH2, in a class of molecules, rather than indicating individual lipids or macromolecules). The most notable feature is that all the correlations observed between small-molecule metabolites and lipids or macromolecules are

negative; i.e. samples with high concentrations of small-molecule metabolites tended to have low concentrations of lipids/macromolecules and vice versa. In the GBM samples there were many negative correlations between lipids/macromolecules and the small-molecule metabolites involved in glycolysis, energy metabolism, membrane metabolism and glutamine and glutamate metabolism. Kizu et al., showed that in the brain tumours after radiosurgery metabolites become undetectable in the necrotic tissue. In some cases, however, macroscopically necrotic tissue showed strong signals from both lipids and choline containing compounds. Opstead et al have shown a positive correlation between signals at 1.3ppm and lipid pseudo-droplets in brain tumour tissues consisting of no-necrosis, low necrosis and high necrosis. The negative correlations observed between the small-molecule metabolites and lipids/macromolecules in this study are therefore probably due to the presence of necrosis, which is a prominent feature of GBM tumours. GBM tissues show numerous negative correlations between metabolites and lipids/macromolecules, probably due to the presence of necrosis.

POSTER 011

Identification of biomarker signatures in pre-clinical glioblastoma patients using metabolomics

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According to Cancer Research UK, primary brain tumors (PBT) has a recorded overall incidence rate of 10-14 cases per 100 000/year, making PBT the 8th most common cancer form in Europe. Glioma accounts for 80% of all PBT and contains cases with varying grades of malignancy (WHO grade I-IV). Glioblastoma multiforme (GBM), is the most common form of PBT, and also the most malignant, with a mean survival time of only 12-18 months. Early detection and diagnosis is a key factor for survival. However, pre-clinical screening programs for early detection of PBT have not been initiated, mainly due to lack of biomarkers. The Janus Serum Bank is a unique population based biobank linked to the Norwegian cancer registry. The samples originate from a total of 317 000 individuals in Norway who have participated in health studies during the period from 1972-2004. Our project aims to investigate alterations in metabolite signatures in serum of healthy blood donors that later on developed GBM. Base line serum from 220 GBM cases, including age, gender, time at serum sampling and storage time matched controls, were obtained from the biobank. Serum analysis was initiated by metabolite extraction in organic solvent followed by comprehensive two-dimensional GCxGC-TOFMS analysis. The obtained mass spectra were processed using the in-house developed 2D-HMCR deconvolution technique for peak extraction. 436 metabolites were detected and 180 metabolites with correct retention index could be identified using reference databases. Metabolites with significant alterations in expression pattern between pre-clinical GBM cases and healthy controls will be presented, evaluated and discussed at the meeting. Nested population-bases metabolomics study identifying metabolite alterations in healthy blood donors, which later in life were diagnosed with GBM.

POSTER 012

Global metabolic profiling of adherent breast cancer cells using GC-MS

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The human metabolome is thought to comprise >100,000 low molecular weight compounds representing a direct signature of metabolic state. Gas-chromatography coupled mass spectrometry (GC-MS) provides the means to detect a wide range of metabolites for the global assessment of metabolic phenotypes. This presents an opportunity to study features of tumour metabolism relating to angiogenesis and metastasis. However, capturing an accurate representation presents a challenge particularly for the evaluation of

mammalian adherent cell types. We applied GC-MS to breast cancer MCF7, MB-MDA-436 and endothelial HMEC-1 cell-lines, cultured in RPMI media supplemented with 0.05% or 10%FBS. Cells underwent detachment with trypsin or a cell scraper before quenching (60% MeOH) and extraction (100% Aq. MeOH at -40°C). Samples were lyophilised and derivatised with methoxyamine and MSTFA (N-methyl-N trimethylsilyltrifluoroacetamide) before being analysed on a TRACE DSQ GC-MS System and identified using the GOLM and NIST databases. Despite a 36% reduction in leakage in trypsinized cells (compared with cell scraped cells), a marked difference in the distribution of metabolic classes were observed, with an approximate two fold increase in the proportion of amino acids and phosphates. This suggests metabolomic fluctuations in response to trypsin. Cell scraping with further modification is therefore recommended for rapid sample preparation. Profiles obtained from cancer cell-lines were more concordant than HMEC-1 and distinct patterns for altered conditions with an approximate 1.5 fold increase in yield for fully confluent and serums starved cells. These preliminary experiments demonstrate the application of GC-MS on adherent mammalian cells; further optimisation is required for future work in detecting metabolic characteristics of tumourgenesis. Cell line dependence of metabolite leakage in metabolome analyses of adherent normal and cancer cell lines

POSTER 013

Candidate Mechanism and Effects of 2-Hydroxyglutarate Accumulation in Human Breast Cancer

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Recently, using a discovery approach and validation of key metabolites, we characterized the metabolomic profile of human breast tumors and uncovered intrinsic metabolite signatures in these tumors. Importantly, the oncometabolite, 2-hydroxyglutarate (2HG), accumulated in a subset of these tumors, reaching mmolar concentrations. Most tumors with high 2HG were of the aggressive basal/mesenchymal molecular subtype. 2HG accumulation correlated with local DNA hypermethylation and a distinct Myc activation signature. In additional work with breast cancer cell lines, we identified the poorly characterized mitochondrial enzyme, ADHFE1, as a candidate source of intracellular 2HG. These observations led us to investigate the oncogenic effects of both 2HG and ADHFE1 in breast cancer biology. Tumorigenic and non-tumorigenic human mammary epithelial cells that are low in endogenous 2HG (e.g., MCF-7, MCF10A and MCF12A), were infected with CMV51p>Hs.AHDFE1-FLAG to generate cell lines overexpressing ADHFE1. Furthermore, ADHFE1 was silenced with shRNA in MDA-MB-231 and SUM159T cells that showed aberrant accumulation of endogenous 2HG. In parallel, we exposed MCF-7, MCF10A, and MCF12A to physiologically relevant concentrations of cell-permeable octylester-2HG (or a mock control octylester). Subsequently, we investigated phenotypic alterations with the following techniques: Proliferation Assay Apoptosis Assay Microscopy (for quantitative analysis of morphologic phenotypes) RNA seq (for differential gene expression analysis, pathway analysis, and Gene Set Enrichment Analysis) Data mining using TCGA data for breast cancer Western Blotting 1. Knockdown of ADHFE1 reduced aberrant accumulation of 2HG to almost baseline level suggesting mitochondrial ADHFE1 as a candidate enzyme involved in the aberrant accumulation of 2HG in breast cancer. 2. A significant change in cell morphology, consistent with epithelial to mesenchymal transition (EMT), was observed in ADHFE1-overexpressing cell lines. Western blot analysis confirmed an increase in expression of EMT marker proteins such as E-cadherin, N-cadherin, Zeb1 and vimentin at the protein level. 3. RNA seq analysis revealed a distinct gene expression profile in ADHFE1-overexpressing MCF-7 and MCF-12A cells. Treatment of breast epithelial cells with 1 mM of octyl-2HG for 48 hours induced a gene expression pattern similar to that of ADHFE1-overexpressing cells. Gene Set Enrichment Analysis further indicated that MCF7 and MCF12A cells overexpressing either ADHFE1 or treated with octyl-2HG have gene expression alterations consistent with EMT. 4. Pathway analysis further showed axonal guidance pathway signaling and regulation of EMT as the top canonical pathways in both ADHFE1-overexpressing cells and cells treated with octyl-2HG. 5. Octyl-2HG treatment significantly enhanced cell

proliferation in MCF7 and inhibited serum starvation-induced apoptosis in both MCF10A and MCF12A cells. 6. Analysis of TCGA data indicated a strong and significant co-amplification of MYC and ADHFE1 in a subset of human breast tumors. Western blot analysis after either knockdown or overexpression of ADHFE1 and c-myc revealed the existence of a feedback loop between ADHFE1 and c-myc. 7. Thus, aberrantly increased 2HG could be at least partly the result of Myc-induced ADHFE1 activation, enhancing EMT and inhibiting apoptosis in human breast cancer cells, consistent with an oncogenic function of 2HG and ADHFE1 in breast cancer. This is the first study showing 2HG as candidate breast cancer oncometabolite and involvement of myc-induced ADHFE1 in 2HG-metabolism.

POSTER 014

Colorectal Cancer Screening and Progression Monitoring Using Targeted LC-MS/MS-Based Metabolic Profiling

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Colorectal cancer (CRC) is the third most common cancer and the third largest cause of cancer death in the US, but is highly treatable when diagnosed in its early stages (Stage I or II). Thus, early CRC detection is the most effective approach to reduce CRC deaths. In addition to the importance of early detection, close monitoring of disease progression (DP) can be critical for patients' prognosis management and treatment decisions. In this study we investigated a targeted LC-MS/MS approach for serum metabolic profiling to detect CRC and to monitor and predict patient therapy, using a panel of significantly altered metabolites as potential biomarkers. This is, as of our knowledge, the first metabolomics-based investigation of CRC disease status surveillance. A targeted HILIC-LC-MS/MS method was developed to measure 164 metabolites across 25 metabolic pathways in serum samples. This method was applied to analyze 234 sera from CRC patients (n=66), polyp patients (n=76) and healthy controls (n=92) as well as 59 serum samples from 21 CRC patients, including 23 samples from DP patients and 36 from other CRC disease status (e.g., stable disease and complete remission). We monitored 106 and 58 MRM transitions in negative and positive mode, respectively. Univariate and multivariate statistical analyses (such as the Mann- Whitney U-test and PLS-DA) were applied for metabolite biomarker discovery and model development on a selected set of promising biomarker candidates. Monte Carlo cross validation (MCCV) was performed to evaluate model robustness. Targeted screening of 164 metabolites from more than 20 different chemical classes (such as amino acids, carboxylic acids, pyridines, etc.) and across 25 important metabolic pathways (TCA cycle, amino acid metabolism, purine and pyrimidine metabolism, and glycolysis, etc.) was performed. 131 metabolites could be reproducibly detected in the serum samples, with an average inter-day CV of 7.1% (2 days) and <11% (12 days). Mann-Whitney U-test analysis of the CRC samples showed that. 42, 48 and 8 metabolites were significantly different ($p<0.05$) in CRC vs. controls, CRC vs. polyps, and polyps vs. controls, respectively. PLS-DA models clearly separated CRC patients from both healthy controls and polyp patients in this study. Receiver operator characteristic (ROC) curves showed high sensitivities (0.96 and 0.89, respectively, for differentiating CRC patients from healthy controls or polyp patients), good specificities (0.80 and 0.88), low false discovery rates (0.22 and 0.14), and excellent AUROC (0.93 and 0.95). Monte Carlo cross validation (MCCV) was also applied, demonstrating the robust diagnostic power of this metabolic profiling approach. For therapy monitoring, univariate analysis showed 36 metabolites, including monosaccharides, amino acids, carboxylic acids and nucleosides, showed differences ($p<0.05$) between CRC DP compared to other disease status (e.g., stable disease and complete remission), and twelve of these were previously reported by other CRC serum metabolites studies. Highly significant changes (defined as $p<0.001$) were found in the average levels of seven metabolites, namely fructose, aspartic acid, oxalic acid, lactate, pyruvate, oxaloacetate and orotate. A PLS-DA model was built based on the combination of these seven metabolite biomarkers, and excellent performance was obtained (sensitivity 96%; specificity 75%) and area under the ROC of 0.92, which was superior to the traditional carcinoembryonic antigen (CEA) marker currently used for CRC monitoring (AUROC=0.76) for detecting

DP over other disease status. This is the first metabolomics-based investigation of CRC disease status surveillance using targeted LC-MS.

POSTER 015

Exploring Metabolic Profile Differences between Colorectal Polyp Patients and Controls Using Seemingly Unrelated Regression

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Colorectal cancer (CRC) is one of the most prevalent and deadly cancers; nevertheless the development of improved and robust biomarkers to enable screening, surveillance, and therapy monitoring continues to be evasive. In particular, patients with colon polyps are at higher risk of developing colon cancer; however, non-invasive methods to identify these patients suffer from poor performance. In consideration of the challenges involved in identifying metabolite biomarkers in individuals with high risk for colon cancer, we have investigated a novel statistical approach to model metabolite levels using nuclear magnetic resonance spectroscopy (NMR)-based metabolite profiling data in combination with numerous demographic parameters to study the ability of serum metabolites to differentiate polyp patients from healthy subjects. A powerful statistical approach, seemingly unrelated regression (SUR), which had not been applied to metabolomics data previously, was used to model the levels of metabolites with demographic parameters. SUR incorporates a correlated error matrix that couples the regression equations. Serum samples from patients with polyps (n=44) and age-matched healthy controls (n=58) were analyzed by NMR. SUR models were built to investigate how the levels of 24 metabolites were influenced by the demographic variables including age, age2, gender, BMI, BMI2, smoking status, alcohol status, diagnosis, as well as the interactions between diagnosis and the other covariates. SUR allows the simultaneous investigation and thus aggregation of disease risk effects on metabolites, and therefore empowers the detection of subtle disease risk effects. Exploratory data analysis showed that several clinical covariates, including gender, BMI, BMI2, and smoking status were significantly associated with the levels of many metabolites. The SUR analysis of all 24 NMR detected metabolites also suggested that metabolite profiles were significantly different between patients with polyps and healthy controls (with a Poster value of 0.0012 for testing diagnosis and its interaction with demographic covariates). Each of the 24 metabolites was then fitted using multiple linear regression to identify metabolites that were significantly associated with diagnosis. Although the levels of valine were slightly different (with an uncorrected Poster value of 0.010), none of the other individual metabolites showed significantly different levels between the patients with polyps and healthy controls as a result of the SUR modeling, indicating the high level of perturbation caused by the confounding factors. However, the analysis of grouped metabolites can enhance the power of hypothesis tests by combining related metabolites in terms of the effects of diagnosis and its interaction with other demographic covariates. We therefore used the KEGG metabolic pathways to map the links among the different metabolites detected by NMR. The SUR results indicated that the effects of diagnosis on the metabolic activities of several groups of biologically related metabolites were indeed quite significant. The groups of metabolites found to significantly distinguish ($p<0.05$) polyps patients from healthy subjects represented numerous metabolic pathways including glycolysis, the Krebs cycle, as well as amino acid and lipid metabolism. Correlation of these metabolites with the detection of polyps patients in this study indicates their potential association with the CRC. Therefore, we developed a SUR modeling approach that combines the results of NMR based metabolomics and the analysis of confounding factors, which has resulted in the identification of a

number of metabolic pathways that are significantly associated with the presence of colon polyps. SUR represents a powerful approach to statistically resolve various confounding factors, which may lead to improved data analysis in metabolomics.

POSTER 016

Metabolomic profiling of chronological effect of imatanib on Gastrointestinal Stromal Tumor (GIST) cells

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GIST is the most common mesenchymal tumor of the gastrointestinal tract. Its progression is commonly driven by mutations in the Kit or PDGFRA genes. Some GIST tumors are effectively treated with Tyrosine Kinase Inhibitors (TKI), which specifically inhibit tumor proliferation by inhibiting their metabolism. Imatinib mesylate, the prototype TKI, has entered the clinical arena in the last decade and substantially improved the outcome in these formerly untreatable cancers. However, many cases develop secondary mutations and resistance. There is an urgent need for novel drugs to treat drug-resistant GIST. The aim of this study is to evaluate the chronological metabolic changes in GIST cells treated with imatinib mesylate and to identify candidate metabolic pathways and metabolites involved in the treatment response. Human GIST-T1 cells were incubated with imatinib mesylate 0.5 mM in triplicates and compared with control untreated cells grown in parallel. Extraction of cell pellets and media at 12, 24 and 48 hours were preformed after viability testing and cell counting. 1H-NMR spectra were acquired using a 500MHz spectrometer equipped with a 5mm TCI 500S2 H-C/N-D-05 Z cryoprobe head at 298 K. A standard one-dimensional Bruker NOESY pulse program with presaturation and spoiler gradients during the relaxation delay, “noesygppr1d.2”. Spectra were further processed in Chenomex NMR suite and metabolites were identified using available libraries. Relative metabolite levels were calculated and compared. Partial Least Squares Discriminant Analysis (PLS-DA) model was used for characterization of chronological biochemical differences in samples. During 48 hours of cell exposure to imatinib, glucose initially increased and then significantly decreased. Untreated cells had steady cell lactate levels over time but with increased media levels, denoting its probable increased production and excretion into the media. When treated, lactate levels decreased in cells and did not increase in media any more. This suggests a shift from cytosolic to mitochondrial glycolysis, as there was a parallel decrease in glutathione, glutamine, glutamate and pyroglutamate levels with minimal changes in pyruvate and succinate. However, glutamine seems to decrease both in cells and media irrespective of treatment, whereas pyroglutamate, similar to lactate, decreases with treatment in cells and doesn’t leak into the media. Cell membrane phospholipids as phosphocholine gradually decreased in the treated cells by a factor of 1.6, suggesting inhibited growth and invasiveness. Glycero-phosphocholine increased in treated cells by a factor of 2.6, while in controls it decreased over time, an observation that needs further studying. Aspartate and taurine gradually increased in cells by a factor of 1.6 and 1.3, respectively; yet that increase was more pronounced in treated cells. Myo-inositol also increases in cells with treatment (factor of 1.7), it does not appear in media. Tyrosine, valine, glycine and leucine showed minimal changes. Cell viability was directly correlated to changes in choline, creatine phosphate, myo-inositol and taurine (correlation coefficient >0.65). PLS-DA model suggested that myoinositol, glycero-phosphocholine, glutamate, aspartate, phosphcholine and glutathione were the metabolites which change in their levels mostly influenced clustering in groups and characterized the differential chronological global metabolic profiles of the cells with continued exposure to imatinib (R2X=0.76, R2Y=0.60, Q2(cum)=0.20). Metabolomic profiling provides new mechanistic and flux insights into longitudinal changes in GIST T1 cells and media exposed to TKI.

POSTER 017

Real-Time Monitoring of Cancer Cell Metabolism and Effects of anAnticancer Agent using 2D In-Cell NMR Spectroscopy

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Altered metabolism is a critical part of cancer cell properties, but real-time monitoring of metabolomic profiles has been hampered by the lack of a facile method. Here, we propose real-time metabolomic monitoring of live cancer cells using 13C6-glucose and heteronuclear two-dimensional NMR. The method allowed for metabolomic differentiation between cancer and normal cells on the basis of time dependent changes in metabolite concentrations. And enabled evaluation of the metabolic effects of galloflavin whose anticancer effects have been attributed to its specific inhibition of lactate dehydrogenase. The method starts with the harvesting of cells grown in normal cell culture media (with 10% serum) and their resuspension in an NMR medium (glucose-free medium with10% dialyzed serum) supplemented with 13C6-labeled glucose.The cells (5 x10^7) were spun in an NMR tube with a weak centrifugal force (30 g for 100 s) to allow sedimentation,enough to cover the active coil region. This step addresses the cell precipitation and the changes in magnetic susceptibility. Then, the NMR tube was put into a spectrometer,and a series of HSQC spectra (total 26; ca. 5 min each)were obtained for the metabolomic study with high sensitivity and confident assignments. Our results revealed metabolic alterations in cancer cells: The cancer cells exhibited much higher production rates for lactate, alanine, and acetate. Intriguingly, these three are the major metabolites synthesized from pyruvate through one biochemical step. Therefore, their higher net production indicates that out-flux of pyruvate is higher in cancer cells than in normal ones. In addition, our results also revealed previously little-noticed metabolic alterations in cancer cells, the increase in the net production of alanine and acetate. Next applied the approach to an investigation of the effects of an anticancer agent, galloflavin, a recently identified lactate dehydrogenase (LDH)-specific inhibitor, especially in the metabolic perspective. The overall pattern of the metabolic shifts was strikingly similar to that between cancer and normal cells,as evident for lactate, alanine, and succinate. These results show that galloflavin not only inhibits pyruvate-to-lactate conversion, but also affects many other metabolic pathways, and that galloflavins anticancer effects seem to be due to these multifacetted effects rather than to specific inhibition of LDH. This highlights the utility of in-cell NMR metabolomics in addressing the possible mechanism of an anticancer agent. Our approach enabled metabolomic comparison of cancer and normal cells and the identification of new targets of an anticancer agent.

POSTER 018

Metabolomic analysis of vitreous humour from retinoblastoma patients

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Retinoblastoma (Rb) is the most common malignant tumor of the eye in children. Inactivation of both copies of the RB1 gene in a child's retina is known to be the cause of cancer. Gene expression microarray studies on patients' cancer tissue samples revealed several pathways e.g. olfactory transduction, cytokine-cytokine receptor interaction, oxidative phosphorylation, phototransduction pathways to be significantly perturbed in patient vitreous samples compared to controls. In this study, metabolomics analysis of vitreous humor was performed to discover differential metabolites in Rb patients which may help unravel underlying molecular pathways driving the cancer. All samples were

collected with prior approval of the Institutional Ethics Committee and informed consent. Vitreous humor samples were extracted with 50% ethanol. Nine patients and two control samples were used. The extracted samples were subjected to LC/QTOF-MS and GC/QTOF-MS analysis. For LC-QTOF analysis, data was acquired using electrospray ionization in positive and negative ion modes using reverse phase C18 and HILIC columns. Molecular features were searched against METLIN database and confirmed by METLIN library. For GC-QTOF analysis, data was acquired using EI source on a DB-5ms column. The results were searched against Fiehn RTL library. All the data acquired from triplicate analysis of the samples is subjected to statistical analysis using Genespring to identify differential features. More than 1000 features were identified using LC/MS and GC/MS techniques. 400 differential compounds were identified between patient and sample groups with a threshold fold change differential of 2 and POSTER value 0.05. A wide variety of compounds including amino acids, carbohydrates, nucleobases, nucleotides, free fatty acids and phospholipids were identified in these differential compounds. All the identified compounds were confirmed by accurate mass and positive and negative mode MS/MS fragmentation pattern. Among lipids, Phosphatidyl cholines (PC), ether linked phosphatidyl ethanolamines (PE), ceramides, sphingomyelins and sphinganines were identified. Lipids, especially PCs and ether linked PEs were found to be 5 folds up regulated in patient samples. Plasmalogens, a subclass of ether lipids containing a double bond and with PE head group were identified and found upregulated in patient samples. Plasmalogens scavenge the free radicals generated by the tumour tissues and offer protection from oxidation. Carnitines that help in lipid transportation in mitochondria were also up regulated 16 folds in patient samples. Biosynthesis of ether lipids starts in peroxisomes and is completed in the endoplasmic reticulum. Hence, dysregulation of peroxisomal metabolites like ether lipids hints at an altered peroxisomal metabolism in these patients. In earlier studies, dysregulation of peroxisomal proteins and PEX genes was reported in connection with cancer. Dysregulated squalene and cholastane triol metabolites reflect the deregulation of cholesterol metabolism associated with Rb. We therefore report a unique metabolic signature associated with Rb mutation driven cancer. Fluids surrounding cancer tissues were used to understand the pathway changes in Rb and further extended to find biomarkers.

POSTER 020

1H-NMR derived metabolomic profile of gastric cancer in urine

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Gastric adenocarcinoma (GC) has a 70-75% mortality due to delayed diagnosis. There is no standard screening in North America, but metabolomic biomarker discovery has shown some promise in early cancer diagnosis. The hypothesis was that metabolomic profiling of urine samples using hydrogen nuclear magnetic resonance (1H-NMR) spectroscopy could discriminate between three disease classes: GC, benign gastric disease (BN), and healthy (HE) patients (pts). Midstream urine samples were collected and biobanked at -80°C from 40 BN, 40 HE and 43 GC pts (matched on age, gender and BMI). A single control patient's sample was sub-aliquoted 17 times and used as a quality control (QC) analyzed every 10th sample. The experiment was run as four single day batches over 10 days. Samples were stratified by class using a random block design. For each urine sample 1H-NMR spectra were acquired using a 600 MHz Varian Inova spectrometer. Metabolite identification and quantification were performed using Chenomx software v7.2. After standard quality assurance procedures 89 metabolites were reproducibly measured (QC-RSD < 25%). Univariate statistics and Multivariate Discriminant Analysis (MDA) were performed to test the proposed hypotheses. There was no significant difference in metabolomic profile between GC and BN. Thirty metabolites differed significantly between GC and HE (Kruskal-Wallis test, $p < 0.05$). OPLS-DA produced a discriminatory model (R-squared = 0.72, Q-squared = 0.56 with one latent variable). A receiver operating characteristic (ROC) curve generated an area under the curve of 0.996. Given a fixed specificity of 100% the corresponding sensitivity was 93% for

discriminating GC from HE. The top five metabolites that contributed to the GC vs HE model were (in order of variable importance (VIP) score): N-acetylglutamine derivative, 3-indoxylsulfate, tropate, sucrose, and 2-furoylglycine. Significant metabolites are involved in gastric mucosal injury, tumour hypoxia, and apoptosis regulatory pathways. This is the first human urine case-control study covering the spectrum of gastric disease (from HE to BN to GC).

POSTER 021

Metabolomics and transcriptomics identify pathway differences between visceral and subcutaneous adipose tissue in colorectal cancer patients – the ColoCare study

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Metabolic and transcriptomic differences between visceral and subcutaneous adipose tissue (VAT and SAT) compartments, particularly in the context of obesity, may play a role in colorectal carcinogenesis. Increased VAT has been strongly associated with metabolic dysfunction and related diseases. While evidence linking obesity and colorectal cancer (CRC) is increasing, the underlying biological mechanisms are still unclear. The colon, which is surrounded by mesenteric visceral body fat, has a direct physical and vascular interface with VAT. Biochemical differences between adipose tissues (VAT vs. SAT) in colorectal cancer patients were investigated using mass spectrometry metabolomics and gene expression profiling. Metabolite compositions were compared between VAT, SAT and serum metabolites. The relationship between patients' tumor stage and metabolic profiles was assessed. This study used specimens from the ColoCare study—a multicenter, prospective cohort of CRC patients that aims to identify strategies for tertiary CRC prevention. Pre-surgery blood and paired VAT and SAT samples collected during tumor-surgery were obtained from 59 CRC patients (tumor stage I-IV). All biospecimens were metabolically profiled using established protocols at the NIH-West Coast Metabolomics Center, Davis; CA. Sampling parameters for adipose tissue were optimized to increase the metabolic coverage for complex lipids. Gas chromatography time-of-flight mass spectrometry (GC-TOF) and liquid chromatography quadrupole time-of-flight mass spectrometry (LC-qTOF) were used to measure 1065 metabolites in adipose tissue (333 identified) and 1810 metabolites in serum (467 identified). Adipose tissue gene expression was measured using Illumina's HumanHT-12 Expression BeadChips. When comparing paired VAT and SAT samples from the same patient, we observed higher levels of almost all amino acids, primary-, glycerosphingolipid- and sphingolipid-metabolism in VAT. Some unsaturated plasmenyl-phospholipid species and phosphoethanolamines were decreased in VAT. Arachidonic acid was higher in VAT but some phospholipid-species containing a 20:4 acyl side chain were lower. We investigated to what extent the serum metabolome was reflective of VAT or SAT metabolism, but only few metabolites were correlated in paired adipose and serum samples: modest correlations (r-Spearman = 0.3-0.4, $p<0.05$) were observed for some triacylglycerols and inverse correlations (r-Spearman < -0.55, $p<0.001$) were observed between serum sphingomyelins and SAT triacylglycerols. We compared SAT and VAT metabolomes of patients with early (I+II) vs. late (III+IV) stage disease using logistic regression and identified 8 VAT lipids which were associated with CRC tumor stage ($p<0.03$). Following our metabolic profiling approach, we further explored the transcriptome of paired VAT and SAT samples, identifying pathway differences in

inflammatory lipid metabolism. Particularly the arachidonic acid pathway, with free arachidonic acid, phospholipases (PLA2G10) and prostaglandin synthesis-related enzymes (PTGD/PTGS2S) was significantly overenriched in VAT compared to SAT. Decreased plasmalogen levels in VAT compared to SAT were supported by a decrease in FAR1 gene expression, the rate limiting enzyme for ether-lipid synthesis in VAT. When correlating patients' BMI with metabolite levels in either VAT or SAT, we observed positive correlations for SAT triglycerides and inverse correlations with phospholipids. Based on this observation we calculated a ratio (total triglycerides /membrane phospholipids) which was positively correlated with patients' BMI in both SAT and VAT. We therefore propose that this ratio may be used as a novel marker for adipocyte cell size. Finally, we visualized our results on a global metabolite pathway level using combined biochemical (KEGG) and chemical similarity (PubChem) networks. Combined metabolomic and transcriptomic profiling identified markers of increased inflammation in human visceral adipose tissue.

POSTER 023

Tracking Aberrant Pathways in Hepatocellular Carcinoma Using Metabolomics: From Tissue Alterations to Blood Biomarkers

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Hepatocellular carcinoma (HCC) is a highly malignant form of liver cancer, with raising incidence and poor prognosis. Heterogeneous phenotypic and genotypic traits, along with a wide range of risks factors, complicate the study of its pathophysiology, and consequently the identification of effective diagnostic markers. Metabolomics can help reveal pathway disruptions due to early sign of cancer through the characterization of thousands of molecules in biological compartments. In this study, we compare metabolite levels in tissue and sera from HCC cases and patients with liver cirrhosis to identify in-situ alterations and track them into the blood stream. Our aim is to find blood biomarkers that are associated to molecular changes observed at the tissue level due to the onset of HCC. We analyzed liver tissues samples and sera collected from 65 participants (40 HCC cases and 25 patients with liver cirrhosis) recruited at MedStar Georgetown University Hospital. In addition to cirrhotic tissues from 25 subjects, both tumor and adjacent non-tumor tissues from each of the 40 HCC cases were receded through biopsy. Thus, a total of 105 frozen dissected tissues were homogenized and subjected to a two-phase sample treatment consisting of extraction of polar metabolites and secondary lipid enrichment. While the polar fractions were analyzed by GC-TOF-MS following derivatization to avoid loss of labile compounds, both fractions were combined for analysis by UPLC-QTOF-MS operating in positive and negative modes. Metabolites extracted from 65 sera were analyzed by both UPLC-QTOF-MS and GC-TOF-MS. In order to be able to evaluate the quality of the metabolomic data we acquired in this study, we spiked the samples with internal standards and quality control (QC) runs. Specifically, debrisoquine and 4-nitrobenzoic acid were used to assess the UPLC-QTOF-MS performance. Also, D35-lysophosphocholine and D7-sphingosine-1-phosphate were included to determine the quality of metabolite extraction. For GC-TOF-MS analysis, the samples were spiked with D27-Myristic acid to verify the derivatization of the compounds. Also, Methyl Stearate was added just before analysis to monitor sample injection. Multiple reference samples obtained by pooling metabolite extracts within each biological group were run in-between the individual samples. These QC runs were utilized to assess the reproducibility of our metabolomic experiments. In addition, the QC runs representing different biological groups were compared to identify metabolites with significant changes in their levels. Peak detection and matching were performed using XCMS, ChromaTOF, and Statistical Compare software tools. For annotation of the analytes detected by UPLC-QTOF-MS, we used MetaboSearch software that we previously developed to retrieve putative metabolite IDs by mass-based search against four databases (HMDB, MMCD, Metlin, and LipidMaps). Analytes detected by GC-TOF-MS were annotated by matching their fragmentation patterns against the NIST, Fiehn, and Golm spectral libraries. Following normalization of the ion intensities, we performed statistical analysis to identify metabolites that showed significant changes between HCC and cirrhotic patients with false discovery rate < 5%. These include metabolites that are associated to Krebs cycle, glycolysis pathways, and energy metabolism. In summary, this study

demonstrates the power of metabolomic analysis of liver tissues and blood samples from the same subjects using two complementary analytical platforms to track pathways altered in tissues and to identify biomarkers circulating in blood. Future studies include validation of these candidates using targeted quantitative methods in sera from independent populations. LC/GC-MS-based metabolomic characterization of HCC to identify aberrant pathways and serological biomarkers associated to molecular changes at the tissue level.

POSTER 024

Proteo-metabolomic dissection of small cell lung cancer using activity based protein profiling and metabolomics profiling

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Small cell lung cancer (SCLC) is poorly differentiated neuroendocrine malignancy which is characterized by poor prognosis and early metastatic dissemination. To date, there are no significant improvements in outcome over platinum-etoposide chemotherapy for SCLC patients. Early detection biomarker strategies and novel therapeutic target discovery is understudied yet remains an important goal. We hypothesized that an assessment of the ATP binding proteome in SCLC coupled with metabolomics could deliver new insights into the disease and nominate both biomarkers and therapeutic targeting strategies. We performed activity-based protein profiling (ABPP) and metabolites profiling in lung cancer cell lines and human tissues. ABPP profiling was performed using desthiobiotin-ATP probe which is directed against the active sites of enzymes.The modified peptides were analyzed by LC-MS/MS and quantified using MaxQuant. The differentially expressed peptides between groups were identified using two-sample Wilcoxon adjusting rank sum test. For metabolites profiling, cell and lung extracts were analyzed by Ultra Performance Liquid Chromatography-Time-of-Flight-Mass Spectrometry (UPLC-TOF-MS). The data were processed using Waters' Progenesis QI software and subjected to statistical and multivariate analysis (e.g., PCA, OPLS-DA) using the SIMCA (Umetrics) software to determine the metabolites that best separate the groups based on inspection of loadings and VIP plots. We profiled the ATPOSTER binding proteome of 18 SCLC and 18 non-SCLC (NSCLC) cell lines. We identified 6937 peptides (2319 proteins), of which 3891 peptides (1543 proteins) were differentially expressed. Several pathways related to metabolism, such as purine biosynthesis and glycolysis / gluconeogenesis, were enriched in SCLC cells compared to NSCLC. These results led us to perform broad spectrum UPLC-TOF-MS metabolomics on ten SCLC and ten NSCLC cell lines. Multivariate analysis demonstrated distinct metabolite profiles for SCLC and NSCLC. Over 100 metabolites with variable importance to projection greater than 1 contributed to the differentiation of the two groups. These included metabolites related to purine metabolism such as inosinic acid and adenosine monophosphate enriched in SCLC cell lines and suggested a connection between our proteomics and metabolomics results. We applied the same approaches to perform the ABPP and metabolite profiling in human lung tissues (7 SCLC, 7 NSCLC and 7 normal lung). ABPP combined with LC/MS/MS identified 17072 peptides (4445 proteins) - 38 proteins are significantly overly expressed in SCLC tissues compared with that of NSCLC and normal tissues. Of these 38 proteins, metabolic enzymes such as ACYP1, CKB, CKM, MTHFD1 and RRM1 were identified. In tissue metabolite profiling, we identified 92 annotated metabolites significantly different between disease (SCLC+NSCLC) and normal tissue. We found 6 metabolites (adenosine monophosphate, 3'-AMP, 8-Oxo-dGMP, Uridine diphosphate-N-acetylglucosamine, Pantothenic acid and FAPy-adenine) enriched in SCLC tissue consistent with our finding at the cell level. In conclusion, ABPP and metabolic profiling identifies distinct enzyme and metabolite profiles in SCLC cells and tissues. Ongoing work aims to integrate ABPP and metabolite profiling using different modeling such as Bayesian network analysis to

jointly characterize the key pathways and constituent components in SCLC. We will update our finding at the meeting. Funded by NIH grants 1R21 CA169979-01A1 (E.B.H) and 1U24DK097193. Integrating proteomics and metabolomics has the potential to identify novel biomarkers and therapeutic targets in diseases.

POSTER 025

Discovery of novel metabolite biomarkers for early diagnosis of lung cancer and monitoring chemotherapy treatment response

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The polyamine pathway has been demonstrated to be significantly uPoster regulated in cancer cells. The enzyme spermidine/spermine N1-acetyltransferase (SSAT1) is recognized as a critical enzyme in the pathway, and is highly regulated in all mammalian cells. While SSAT1 is present in normal tissues in very low concentrations, it is present at much higher levels in cancer cells. Therefore, as cellular levels of SSAT1 increase, measurement of its enzymatic activity correlates with the presence and severity of cancer. Here we demonstrate the utility of measuring polyamine pathway metabolites as a tool for early detection of lung cancer and for monitoring chemotherapy treatment. An MS-based assay was developed to simultaneously detect and quantify specific polyamine pathway metabolites. DI-MS was also performed via Biocrates Absolute p180IDQ Kit. Metabolites were quantified in serum and urine samples from 50 lung cancer patients and 50 control patients, and in serum and urine samples from lung cancer patients undergoing chemotherapy treatment. Morning urine samples were collected from 30 patients during two chemotherapy treatment cycles (approximately 3 weeks apart). Four metabolites have been identified as putative biomarkers for early lung cancer (the specific metabolites are not named at this time for confidentiality purposes). The results from this study revealed a preliminary picture of the polyamine metabolome in cancer patients and healthy subjects. ROC analyses demonstrates the potential utility of these four metabolites as biomarkers for cancer diagnosis and treatment prognosis. Polyamine quantification and SSAT1 enzymatic activity provide a novel route for detecting early lung cancer and monitoring chemotherapy treatment.

POSTER 026

Metabolic reprogramming in lung cancer

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Lung cancer's very low 5-year survival rate makes it the most common cause of cancer death worldwide. Current treatments, many of which use platinum-based chemotherapies, arecharacterized by great heterogeneity both in terms of efficacy and toxicity, and their long-term effectiveness is generally very limited. Metabolic reprogramming is considered an essential hallmark of cancer, and therefore the study of lung cancer metabolic reprogramming can be an effective tool to detect enzymes to be targeted in order to block the metabolic pathways that cancer cells need to sustain their accelerated proliferation rates. The detection of such enzymes and the validation of their potential as anti-tumor targets can help define new therapeutic strategies able to improve the efficacy of current treatments. In this study we have combined classical biochemical measurements and tracer-based metabolomics measurements using the 13C labelled substrates [1,2-13C]-glucose and [3-13C]-glutamine, in order to study the differences in

central metabolism between the KRAS-mutated lung cancer cell lines A549 and NCIH460, and the non-tumor lung cell line BEAS2B. All three cell lines were incubated with the 13C labelled substrates for 24h, and cells were counted before and after the incubations. Biochemical measurements were carried out in cell media by spectrophotometry, and Isotopolog distribution of several metabolites was measured by Gas Chromatography-Mass Spectrometry (GC-MS). Information derived from mass isotopomerdistribution analysis and biochemical measurements was processed computationally in order to compare the metabolic flux profiling in cancer and non-cancer cell lines. Results indicated higher consumption rates of glucose and glutamine in the lung cancer cell linesA549 and NCIH-460 in comparison with the non-tumor cell line BEAS2B, with higher production rates of lactate and glutamate. Differences have also been observed at the level of the metabolism of pentose phosphate pathway between the tumor and the non-tumor cell lines.Moreover, mass isotopomer distributions indicate that glutamine is used as a carbon source to synthesize fatty acids in the tumor cell lines, suggesting the existence of activated cancer-related glutaminolytic pathways. In sum, results suggested the existence of strong glutamine dependence in both tumor cell lines, which could be an interesting target to be exploited in cancer therapy. Study of lung cancer metabolic reprogramming has allowed us to identify a possible metabolic target exploitable to impair tumor proliferation.

POSTER 027

Vitamin D Associations with Lung Cancer in a Case-Control Study

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Vitamin D is an essential micronutrient, required for normal physiological function and classically recognized for its role regulating calcium metabolism. Additionally, recent work is beginning to demonstrate a role of vitamin D in chronic illnesses such as cancer, but questions addressing how depletion of this nutrient affects cancer risk remain largely unanswered. Previous work in our laboratory addressed whether there are biomarkers associated with lung cancer risk and survival that are detectable in urine. We uncovered metabolites associated with both lung cancer risk and survival, measured in both urine and tumor tissue, and among those identified was a potentially related vitamin D3 metabolite. Our previous urinary metabolomics study prompted us to measure circulating serum vitamin D levels in relation with lung cancer risk and survival in a large study comprising 434 cases and 403 controls. Using targeted ultraperformance liquid chromatography coupled to mass spectrometry (UPLC-MS), we undertook a first of its kind effort to quantitate both inactive, 25(OH)D, and active, 1,25(OH)2D forms of both, vitamins D2 and D3, in the serum of a large number of subjects included in the study. We discovered that high levels of 25(OH)D3 are associated with lower lung cancer risk across quartiles, after adjustment for age, gender, race, smoking status and pack years (ORadjusted ranging from 0.50 to 0.46; $P < 0.0001$). The associations were observed as stronger in African Americans when compared to European Americans (ORadjusted = 0.23 compared to 0.51 in the highest quartile, respectively). We did not observe significant associations of 25(OH)D2 with lung cancer risk, a form of vitamin D that predominantly comes from exogenous sources, such as plants. Importantly, when we stratified samples by months when the blood was collected (non-sunny months: November – April, vs. sunny-months: May – October), we observed a significant association between high levels of 25(OH)D3 and lung cancer in individuals whose blood was collected during the non-sunny months, when potential confounding due to sun exposure is less prominent. Most importantly, we measured 1,25(OH)2D2 and D3, active forms of vitamin D, in a subset of 100 subjects; active form of vitamin D has been shown to have anti-proliferative properties in the context of carcinogenesis in many different cancer types. We observed a significant association between high levels of 1,25(OH)D3 and lower lung cancer risk across quartiles (ORadjusted ranging from 0.34 to 0.13, $P < 0.0001$). We are in the process of completing the analysis of a comprehensive number of 299 SNPs in the vitamin D metabolizing enzymes (cytochrome P450 and vitamin D receptor genes), wherein we are considering that some polymorphisms may modulate the levels of vitamin D, and that they might be associated with lung cancer risk; these results could uncover biological factors that contribute to the health disparities in lung cancer. This research may illuminate a relationship between vitamin D and cancer and the importance of optimal levels for human health.

POSTER 028

A Systematic Analysis of Bidirectional Metabolomics Changes upon Interaction of Cancer Cells with Adipocytes

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Metastasis is the primary cause of death in ovarian cancer (OvCa) and the most common site is the omentum, a large fat pad (20x10x3cm) protecting the abdomen. The omentum is an endocrine organ that is primarily composed of adipocytes and represents a unique tumor microenvironment, rich in lipids, cytokines and eicosanoids. We have previously shown that OvCa cells induce lipolysis in adipocytes and enhance the uptake of lipids, thereby augmenting cellular proliferation. However, how cellular metabolism (a hallmark of transformed cells) is altered in the metastatic microenvironment has not been studied. Hence, we hypothesized that OvCa cells metastasizing to the omentum have altered intracellular signaling and undergo metabolic reprogramming that contributes to metastatic spread. To mimic the dynamic interaction between adipocytes and OvCa (SKOV3ip1) cells in metastasis, we co-cultured primary human adipocytes (isolated from cancer-free donors) with OvCa cell lines, using an organotypic 3-D model. After 18 hours, we separated adipocytes from cancer cells and collected cell pellets and tissue culture media. We carried out an unbiased metabolomics analysis using GC and LC MS platforms to identify primary metabolites, complex lipids and oxylipins. To identify altered cellular signaling and protein expression, we performed Western blot and qPCR analysis using cancer cells that had been co-cultured with primary human adipocytes. In addition, we used a xenograft mouse model, inhibiting target proteins to evaluate their contribution to the metastatic process. Metabolomic analysis of adipocytes and OvCa cells in co-culture revealed alterations in both cell types and in the culture media. With the reduction of fatty acids (such as palmitic and stearic acid) in the adipocytes, there was a concomitant increase in the accumulation of neutral lipids, such as triacylglycerol, in the cancer cells, suggesting the dynamic exchange of cellular metabolites between the two cell types. We also found increased levels of membrane lipids, such as phosphatidylcholine (PC) and phosphatidylethanolamine (PE), in the cancer cells. This suggests a possible increase in the de novo synthesis of structural lipids and increased membrane synthesis, which is consistent with the enhanced cancer cell proliferation observed with adipocyte co-culture. Concomitant with an increase in acyl carnitine, intracellular lipid accumulation, and decreased free carnitines, we observed increased β-oxidation in SKOV3ip1 cells on co-culture. We also observed profound changes in the co-culture media, with increases in oxylipins, such as pro-inflammatory prostaglandins (PGE2, PGE3, and TXB2), which are known to regulate cellular proliferation. In order to identify the causative mechanisms regulating changes observed in the metabolome, we examined the role of proteins involved in fatty acid metabolism. We observed that, in the presence of adipocytes, cancer cells enhanced lipid utilization machinery with the transcriptional upregulation of CD36 protein, a fatty acid transporter molecule at the cell membrane, and fatty acid binding protein 4 (FABP4), an intra-cellular lipid chaperone. Pharmacological or siRNA mediated inhibition of these proteins led to the dramatic reduction of intracellular lipid accumulation and β-oxidation. Moreover, inhibition of these proteins led to the significant reduction of tumor burden in a xenograft mouse model. In summary, omental adipocytes induce metabolic changes regulating intracellular lipid accumulation, potentially increasing the metastatic potential of OvCa cells. A 3D-culture of primary adipocytes and OvCa cells was used to elucidate bidirectional metabolomic changes in cancer and stroma.

POSTER 030

Phenotyping of early stage ovarian cancer by mass spectrometry untargeted metabolomics

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Lack of symptoms as well as the deficiency of highly specific biomarkers has resulted in only a quarter of ovarian cancer (OC) cases being diagnosed at stage I. Early detection combined with conventional therapies has resulted in 5-year survival rates up to 90%, while 5-year overall survival is less than 30% for women with advanced-stage OC. Investigation into characteristic metabolomic patterns for disease has the potential to detect changes in cells, tissues, and biofluids that can aid in early-stage diagnosis. Serum samples were collected from early-stage papillary serous or endometrioid epithelial ovarian cancer (EOC) and normal patients, and analyzed using ultra performance liquid chromatography coupled with high resolution mass spectrometry (UPLC-MS) and tandem mass spectrometry (MS/MS). Metabolites were extracted from blood serum by precipitating proteins with methanol, lyophilization, and solvent reconstitution prior to MS analysis in negative electrospray ionization mode. Metabolic features were extracted with MZmine software. Untargeted multivariate statistical analysis employing support vector machine (SVM) learning methods and recursive feature elimination (RFE) selected a panel of metabolites that differentiates between the age-matched samples. Comparison of metabolic phenotypes of EOC with normal metabolic signatures revealed unique metabolite patterns for EOC in studies of two different patient cohorts. The first study compared early-stage papillary serous or endometrioid EOC (n=24) and normal patients (n=40). As papillary serous is the most commonly diagnosed histopathological subtype of EOC, a second study compared only early-stage papillary serous EOC (n=46) and normal patients (n=49). From multivariate statistical analysis, panels consisting of 16-22 metabolic features from serum samples were found to differentiate between early-stage EOC and normal with very high accuracy, sensitivity, and specificity. The dominant classes of metabolites in the panels were lipids and fatty acids; this correlated well with the literature in which the metabolomes of EOC patients exhibit disruption of lipid metabolism and profiles. Poor early diagnosis complicates collection of large patient cohorts for more detailed studies. Our preliminary work demonstrated that metabolites in serum samples are useful for detecting early-stage EOC and support conducting larger, more focused studies. First MS-based metabolomic study of early-stage EOC serum focused on biomarker discovery for early detection.

POSTER 031

Metabolomics of Exosomes from Uterine Aspirates and Plasma Samples of Endometrial Cancer Patients

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Endometrial cancer is the most frequent gynecological cancer diagnosed in the United States, accounting for 76,000 deaths annually. Mortality is associated with presentation of poor prognostic factors and/or advanced disease at diagnosis. Exosome-like vesicles (ELV) obtained from easy-to-access biofluids are an untapped resource for discovery and validation of clinically relevant biomarkers in health and disease. Although the proteomic and transcriptomic profile of ELV have been broadly described, the metabolomic profile is uncharacterized. Thus the goal of this study is to characterize the metabolome of ELV derived from human uterine fluids and plasma, and identify metabolomic signatures that could be used for risk stratification and diagnosis of endometrial cancer patients. Uterine aspirate and plasma samples from 5 endometrial cancer and 8 benign patients were collected and processed in Vall Hebron Hospital (Barcelona, Spain) in accordance with approved institutional consent and review protocols. ELVs were isolated by standard ultracentrifugation, characterized by immunoblot against known exosomal markers and size and concentration estimated using Nanoparticle Tracking Analysis. We analyzed the metabolomic and lipidomic profile of the ELVs by ultra-performance liquid chromatography coupled with electro-spray quadrupole time of flight mass spectrometry (UPLC-ESI-QTOF-MS). Data were pre-processed using the XCMS software while the database search was performed using the Madison Metabolomics Consortium Database (MMCD), the Human Metabolome Database (HMDB), LIPID MAPS and Metlin for putative metabolite identification. As a first step, we optimized the starting volume of biofluid for exosome isolation that would yield high spectral data quality. Thus, ELVs from different starting volumes ranging from 200 to 800µL of uterine fluid and from 250 to 2000µL of plasma were isolated, and confirmed by determining the expression of known exosomal markers Flotilin 1, TSG101, CD63, CD9, Rab5, and CD81. The average size of the exosomes was found to be 140nm±10nm. Comparative analyses showed that 400µL of uterine aspirates and 250µL of plasma as a starting was sufficient for ELVs isolation to produce high quality metabolomic and lipidomic data. The metabolome and lipidome of ELVs derived from uterine fluid and plasma samples contained a high percentage of phospholipids, peptides and nucleotides within ELVs, and interestingly, some other less common molecules such as Vitamin D derivatives. A comparative analyses of metabolites derived from plasma and uterine aspirate exosomes suggested unique features for each matrix although a number of metabolites were common to both matrices. These findings once relocated could have broad application for clinical and translation studies that focus on biomarker development. Furthermore, a metabolomic and lipidomic profile that differentiates endometrial cancer from benign patients was obtained; validation of these findings is ongoing. This is the first report that attempts to examine the metabolomics profile of uterine aspirates and plasma exosomes.

POSTER 032

Metabolomic investigation of tamoxifen induced endometrial cancer

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Approximately 230,000 new breast cancer cases occur annually in the US, and of these cases 50-80% are hormone receptor positive (ER+). One common prescribed treatment for ER+ breast cancer is Tamoxifen (TAM). TAM reduces the risk of reoccurrence by 50%, and decrease the risk of death by 30% in women with ER+, early stage breast cancer. However, one side-effect of TAM is a 3 fold increased risk of developing endometrial cancer, and the mechanism behind this increase is not known. To get a mechanistic insight, we have explored TAM metabolism in vivo and in vitro and report a new metabolic pathway of TAM that could induce endometrial cancer. Waters Acquity UPLC system connected to the Xevo-TQ triple-quadrupole mass spectrometer (UPLC-MS/MS) was used in all studies. Standards were purchased or synthesized and used to optimize the UPLC–MS/MS conditions before analysis. Urine from postmenopausal women taking TAM was collected and TAM metabolism was investigated in vivo using

UPLC-MS/MS based metabolomics technique. Furthermore, we performed horseradish peroxidase (HRP) assays to confirm peroxidase involvement in TAM metabolism in vitro. Substrates, 4-hydroxytamoxifen (4-HT), endoxifen, or bisphenol (BPT), were added to buffer solution containing HRP and H2O2 and the assay mixtures were incubated for 30 mins, filtered, and aliquots were profiled using UPLC-MS/MS. The resulting data from all experiments were analyzed and processed using MassLynx 4.1 software. Profiling of human urine for TAM metabolites using UPLC-MS/MS identified a hitherto unknown metabolic pathway of TAM, in addition, major metabolites reported earlier were also identified. Specifically, urine from a postmetapausal woman taking TAM showed the formation of BPT-Ade, suggesting the metabolic activation of TAM to BPT and further, its covalent interaction with adenine to form BPT-Ade adduct in vivo. UPLC-MS/MS analysis of the assay mixtures indicated that peroxidase carry out side chain cleavage of 4-HT to yield BPT. Moreover, BPT undergoes peroxidase catalyzed oxidation to form a reactive quinone methide. When adenine was included in the assay mixture, the quinone methide alkylated it to form BPT-Ade adduct. In summary, our in vitro and in vivo results suggest that TAM is metabolized to BPT which further forms quinone methide through mediation of peroxidase. BPT-quinone methide covalently interacts with DNA to form adducts. This adduct depurinates to form apurinic sites on DNA that can lead to mutations and cancer. Metabolomic investigation suggests a new TAM metabolic pathway in women that could lead to endometrial cancer associated with TAM administration.

POSTER 034

Predicting resectability of pancreatic cancer using GC-ToF-MS and UHPLC-MS metabolic profiles from urine.

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Pancreatic cancer (PC) is one of the most lethal types of cancers with the majority of patients suffering from irresectable tumours at the time of diagnosis. Surgery is usually the only way PC can be completely cured. The ability to ascertain the chances of the tumour being removable by surgery is thus essential. Current biomarkers fall short in accessibility, sensitivity and specificity and their ability to distinguish malignant from benign conditions. Metabolomics aims to decipher molecular signatures that will distinguish disease from healthy physical state, ultimately leading to novel targets for diagnosis and treatment. The study aimed to (a) characterise potentially new PC biomarkers by comparison PC cases to healthy controls (HC); and (b) evaluate markers for tumour resectability. Urine samples from collected from pancreatic cancer (n=28) and healthy controls (n=36) were analysed with GC-ToF-MS and reverse phase UHPLC-Orbitrap MS to resolve and identify polar and non-polar metabolites respectively. Raw data were deconvolved using XCMS based on in-house R scripts. Peaks intensities were aligned with the LOESS algorithm and those <20% RSD in pooled QCs were removed. The resulting data matrices were subjected to principal component-discriminant function analysis and the Student's t-test to find important variables that (a) separate PC cases from healthy controls; (b) separate resectable and non-resectable pancreatic cancer patients. GC-MS fragment spectra were matched with in-house and NIST libraries for identification of analytes, LC-MS accurate masses were matched against METLIN, HMDB and KEGG databases. Resectability of PC: Using a hybrid PC-DFA classification algorithm, resectability was predicted with 100% accuracy and these models were validated using 1000 bootstrap cross-validations. The differences between these two groups were so clear that PCA analysis was sufficient to visualise group separation. Importantly there did not seem to be any effect from diabetes or jaundice on the differentiation between the resectable and non-resectable tumours. Comparing PC vs. HC: After univariate analysis of combined data from both analytical techniques, 558 urinary features were found to be significantly different between PC and HC (Poster value < 0.05) whereas 48 urinary features were found to be significantly different between resectable and non-resectable PC (Poster value < 0.05). The significant threshold of Poster values was adjusted using false discovery rate. Some of the key analytes

putatively identified to be differential of PC have been previously suggested to be linked with pancreatic cancer. However, in the current study work is being undertaken to map specific pathways involved in altering these analytes significantly in PC subjects. Nine analytes were found to not only differentiate between PC and HC but also, between resectable and non-resectable tumours in pancreatic cancer subjects. 3-Amino-1-methyl-5H-pyrido [4, 3-b] indole, a well-known carcinogen was found elevated in subjects with non-resectable PC compared to those with resectable PC as well as HC. Aspartate levels were also found to be higher in HC but lower in PC with resectable tumours. Some groups have suggested increased levels of aspartate in tumour tissues, however its direct link to PC is still unknown. These preliminary results indicate that metabolomics can be successfully employed to (1) confirm diagnosis of PC by distinguishing PC from HC; and (2) aid the important clinical decision of resectability of pancreatic cancer. Resectability of tumour in pancreatic cancer can be predicted with 100% accuracy using metabolic biomarkers identified in urine.

POSTER 035

Bcl-2 protein Noxa regulates cellular energetics and reveals novel pathways in cancer metabolism

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Cancer cells increase the consumption and metabolism of both glucose and glutamine and reprogram metabolic pathways to enhance biomass production. Glucose is often diverted to alternative pathways for the production of building blocks prior to the generation of pyruvate and lactate. However, a high glycolytic rate needs a steady supply of cytoplasmic NAD to be regenerated from NADH, a task that has historically been attributed to lactate dehydrogenase. This suggests that alternative pathways of NAD regeneration are being utilized. Furthermore, increased glutamine uptake and metabolism create an additional problem. The first two steps of glutaminolysis generate two molecules of free ammonia. How cancer cells mitigate the potential toxicity from this ammonia is currently unknown, and of immense therapeutic value. To address these questions, we used a T cell leukemia model in which over-expression of the pro-apoptotic Bcl-2 protein Noxa increases glucose consumption, extracellular acidification and proliferation. Noxahi cells metabolize the glucose through anabolic routes, including the pentose phosphate pathway. Thus, these cells represent a valuable tool for investigating metabolic adaptations in cancers. First, we carried out metabolic tracer analysis, with a [2H] labeled glucose that transfers the [2H] to NADH during glycolysis, to determine how Noxahi cells regenerate NAD. We also used [13C]-U-glutamine to trace its path via glutaminolysis and 15N-amide-glutamine to investigate the fate of free ammonia generated during glutaminolysis. Metabolites were identified using a combination of liquid chromatography and/or gas chromatography coupled with mass spectrometry. Tracer studies with [2H] glucose revealed that Noxahi cells prefer malate dehydrogenase to lactate dehydrogenase for regenerating cytoplasmic NAD. Tracer studies with [13C]-U-glutamine showed that the reductive carboxylation of glutamine is a significant source of malate in these cells and is able satisfy the demand for cytoplasmic malate for NAD regeneration. Unexpectedly, the experiment with [2H]- labeled glucose also revealed a significant increase in [2H] labeled aspartate in Noxahi cells. This aspartate could potentially be generated from early glycolytic intermediates or from a reaction that required NADH as a cofactor. To date, we have been unable to identify a pathway that would yield significant amounts of deuterated aspartate from glycolytic intermediates at 24 hours. There is, however, a reaction that requires NADH to produce aspartate. The enzyme aspartate dehydrogenase (ASPDH) utilizes the reactive hydrogen from NADH to generate aspartate from oxaloacetate. This reaction also utilizes free ammonia from the environment for the amine group of aspartate. As stated earlier, how cancer cells mitigate the potential toxicity that results from accumulation of free ammonia generated during glutaminolysis is currently not known, but is of much therapeutic interest. Our data suggest that aspartate from the ASPDH catalyzed reaction is exported out of the cell, contributing to the increased acidification. This pathway may represent a novel mechanism to package and excrete excess ammonia. We hypothesized, based on these observations, that siRNA targeted to ASPDH would decrease proliferation and/or promote cell death. We also hypothesized that any cancer cell type exhibiting dependence on glutamine should be

sensitive to loss of ASPDH. Colorectal cancer cells transiently transfected with ASPDH siRNA transfected displayed significant decrease in cell viability and proliferation compared to control siRNA transfectants within 72 hours. Preliminary studies also showed an increase in detectable free ammonia in cells treated with ASPDH siRNA. Our studies indicate that cancer cells utilize a novel ASPDH catalyzed step for regenerating cytoplasmic NAD and counteracting ammonia toxicity.

POSTER 036

MCT1-dependent lactate utilisation is a "metabolic escape" pathway that maintains tumour cell proliferation under glucose deprivation

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Malignant cells within solid tumours can be frequently exposed to microenvironments where glucose is lacking while simultaneously having access to high levels of an alternative substrate, lactate. Lactate utilisation is dependent upon the moncarboxylate transporter MCT1 and can be targeted by the specific clinical MCT1 inhibitor AZD3965. We hypothesised that lactate utilisation via MCT1 is an adaptive response to glucose deprivation or drugs that inhibit glycolysis. Reducing the ability of tumour cells to escape nutrient deprivation could be an important route to blocking disease progression and the acquisition of drug resistance. Using a combination of specific interference with MCT1, metabolomics, stable-isotope labelling experiments, metabolic modelling and protein assays, we have investigated the response of cancer cells to glucose deprivation in the presence of lactate. We found that in the absence of glucose, the presence of lactate was sufficient to maintain growth of solid tumour cells across several lineages. Imported lactate was converted to pyruvate and enriched the TCA cycle. rescuing the ATP depletion observed under glucose deprivation. Lactate also protected against oxidative stress caused by glucose withdrawal maintaining NADPH/NADP+ and GSH/GSSG. Under lactate supplementation/glucose deprivation carbon from lactate enriched a high proportion of fatty acids and (~60%) of the lipogenic acetyl CoA pool indicating that it contributes significantly to lipid anabolism. AZD3695 suppressed de novo lipid synthesis under these conditions supporting this hypothesis. We also identified conversion of lactate into PEP, 3PG, DHAP and glycerol-3-phosphate (G3P) suggesting entry into gluconeogenesis and glyceroneogenesis. Lactate metabolism under glucose deprivation correlated with protein-level increases in MCT1 and LDHB with concomitant repression of LDHA, suggesting an active adaptation of cells to favour import of lactate and conversion to pyruvate. We also detected upregulation of the transcriptional co-factor PGC1a under these conditions suggesting that it may act as the upstream regulator of lactate metabolism as is observed in normal physiology. Lactate supplementation was able to protect cancer cells from the growth inhibitory effects of glycolysis inhibitors such as 2-DG which phenocopy glucose deprivation. This protection was lost upon co-treatment with AZD3965 suggesting a rational strategy for combining clinical MCT1 inhibitors with other chemotherapeutics that cause a reduction in glycolysis to increase efficacy and prevent drug resistance via metabolic escape. Blocking metabolic escape via lactate utilisation is a new therapeutic strategy that could reduce resistance to existing anti-cancer agents

POSTER 037

Decoding the response of cellular metabolism to the action of glycolytic inhibitors using pulsed stable isotope-resolved metabolomics

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Since the observation that cancer cells rely heavily on glucose as their main carbon source the elevated glycolysis serves as one potential target for cancer treatment and several inhibitors have already been described in the literature. However, the mechanisms of their inhibition are often not well understood. Thus rational targeted strategies for a metabolic cancer therapy are limited. In order to better understand the action of proposed inhibitors of glycolysis and to build a strategy to identify the action of yet unknown compounds, we performed a variety of experiments on different timescales. The basic principle is to measure the impact of compounds on glycolysis, on a short timescale in combination with stable isotope

tracing techniques. We incubated human cell lines with different compounds supposed to inhibit glycolysis, in a timeframe short enough to detect the direct inhibition on the enzyme level before regulatory networks could induce changes in the enzymatic composition of the cell. Within this timeframe we additionally fed 13C-glucose to the cells, to monitor the flux through glycolysis. Ultimately, we measured intracellular metabolites within glycolysis and closely connected pathways by GC-MS, determining both their pool sizes and the 13C-incorporation in parallel. The results show that the 13C incorporation data offer an additional and important layer of information, as the impact on the pool size was quite small even under conditions where the 13C-flow broke down nearly completely. Surprisingly we recognized that 2-deoxyglucose offers only a limited potential to inhibit glycolysis directly and may act primarily through the depletion of the intracellular ATP and phosphate pools. In contrast, 3-bromopyruvate as well as other tested compounds were acting as potent glycolytic inhibitors. These results show that it is indeed important to discriminate early direct effects from subsequent secondary effects. In addition, the position of the metabolic block within the network can be narrowed down with this approach. We think that this strategy will help to deliver a framework for improving metabolic therapies. With this approach it is possible to discern primary and secondary metabolic responses to drug or inhibitor treatments.

POSTER 038

LC/MS and GC/MS urine metabolic fingerprinting in prostate cancer

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Prostate cancer (CaP) is a leading cause of cancer deaths in men worldwide. Although the alarming statistics, the currently applied biomarkers are still not enough specific and selective. In addition, pathogenesis of CaP development is not totally understood. Therefore, metabolomics study related to urinary metabolic fingerprinting analyses has been performed in order to scrutinize potential biomarkers that could help in explaining the pathomechanism of the disease and be potentially useful in its diagnosis and prognosis. To the best knowledge, this report is the first regarding usage of two complementary analytical techniques in urinary untargeted study of CaP. The advantage of applying LC and GC techniques is the possibility of determination of broader set of metabolites than using only one technique. Urine samples from CaP patients and healthy volunteers were analyzed with the use of high performance liquid chromatography coupled with time of flight mass spectrometry detection (HPLC-TOF/MS) in positive and negative polarity as well as gas chromatography hyphenated with triple quadruple mass spectrometry detection (GC-QqQ/MS) in a scan mode. The obtained data sets were statistically analyzed using univariate and multivariate statistical analyses. The Principal Component Analysis (PCA) was used to check systems' stability and possible outliers, whereas Partial Least Squares Discriminant Analysis (PLS-DA) was performed for evaluation of quality of the model as well as its predictive ability using statistically significant metabolites. R2 and Q2 factors were calculated based on 7-fold cross-validation approach. After applying QA criteria, the datasets consisted of 677 variables for LC-TOF/MS analysis in positive ionisation mode, 414 compounds in negative ionisation mode and 41 variables obtained after GC-MS analyses. Those datasets were used to build PCA models. Good clustering of QC samples and no outliers according to Hottelling T2 range were observed for both LC-ESI-TOF/MS and GC-MS PCA models. For LC-TOF/MS analyses in positive ionisation mode 235, for negative ionisation mode 248 and for GC-MS analyses 28 statistically significant variables were selected. Partial Least Squares Discriminant Analysis models were built on normalised intensities (MSGUS approach) of statistically significant variables. In case of LC-TOF/MS data set obtained in positive ionization mode, the R2 and Q2 were 0.756 and 0.579, respectively, whereas in negative ionization mode these two values were set as 0.763 and 0.508, respectively. Moreover, for GC-MS data set, the R2 and Q2 were set as 0.788 and 0.711, respectively. The subsequent identification of selected metabolites using NIST library and commonly available databases allows for creation of a list of putative biomarkers and related biochemical

pathways they are involved in. The selected pathways, like urea and tricarboxylic acid cycle, amino acid and purine metabolism, can play crucial role in pathogenesis of prostate cancer disease. The results presented herein, indicate that disruption of amino and organic acids as well as carbohydrates metabolism may be specific for prostate cancer metabolic phenotype which can be also associated with abnormal cell growth and intensive cell proliferation. It is the first report on usage of two complementary analytical techniques in urinary untargeted study of CaP disease.

POSTER 039

Identification of new drug target candidates for prostate cancer and heart failure by a systems biology approach

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dentification of new drug targets is important for modern pharmacology. The sequencing of the human genome and several pathogen genomes suggest that there are more potential drug targets than the approximately 500 reported until 2000 [Drews, Science 2000, 287:1960-1964]. The estimated 20,000 to 25,000 protein-coding genes harbor a reservoir for new drug target identification. However, how to extensively use this source is a challenge. A systems biology approach, meaning an –omics data integration could help to identify new drug target candidates. Metanomics Health obtained metabolomics data by a GC-MS and LC-MS/MS based MxP® Broad Profiling platform of (a) human prostate cancer and adjacent normal tissue as well as from (b) myocardium of a mouse model of heart failure (transversal aortic constriction including sham-operated control). Metabolomics data of (a) prostate cancer was integrated with public transcriptomics data and of (b) heart failure was integrated with transcriptomics data from the same samples. Data integration was done by relating metabolites to protein function of the corresponding gene. New drug target candidates were nominated by applying different filter strategies. Genes of potential interest in prostate cancer therapy were further investigated in a siRNA cell culture model system for gene silencing and phenotype characterization. For prostate cancer tissue, the metabolomics data set comprised 172 known metabolites and the transcriptomics data set comprised >15 000 transcripts. 113 metabolites and 2112 transcripts could be integrated into a systems biology data set using the related protein-metabolite function[PT1] . For myocardium tissue, the metabolomics data set comprised 209 known metabolites and the transcriptomics data set comprised >45 000 transcripts. 161 metabolites and 2559 transcripts could be likewise integrated into a systems biology data set. Using different filter approaches, 7 and 16 transcripts were nominated as potential new drug target candidates for prostate cancer and heart failure, respectively. Using a siRNA cell culture model system, three genes out of the 7 nominations were validated to be effective in inhibition of prostate cancer cell lines and present a valuable new finding in treatment options and elucidation of metabolic aspects of pathological mechanism of the disease. With respect to heart failure, cytidine triphosphate synthase 1 (CTPS1) was identified by the same approach and validated as positive control. To prove the value of the systems biology approach, we compared the numbers of known drug targets (proof of concept findings for heart failure) identified from the systems biology data set to the number from the transcriptomics data set when applying the same filter strategy. Fisher's exact test showed a significant enrichment with Poster value <0.05 when using the systems biology data set. We concluded that data integration of two –omics data sets reduces the number of potential candidates needed to be screened in order to find promising hits and therefore results faster in valuable new drug targets. [PT1]Verständlicher ausdrücken, z.B. "using

metabolite–protein relationships imported from the Human Metabolome Database (HMDB)" Metabolomics is a valuable key in systems biology for identification of new drug target candidates and elucidation of pathological mechanisms.

POSTER 040

An integrated evaluation of metabolomics, transcriptomics, and immunohistochemistry data

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Prostate cancer (PCA) continues to be the most frequent cancer in men. The developments in "-omics" technologies such as genomics, transcriptomics, proteomics, and metabolomics provide a promising basis for research efforts to better understand the underlying molecular, physiological, and biochemical processes of tumor initiation and progression. Metabolomics complements transcriptomics and proteomics as a physiological "down-stream result" of the –omics cascade thus being closer to the phenotype and providing a more comprehensive overview of a cancer cell's metabolism. Moreover, an integrated view in combination with protein expression data and gene expression data (systems biology approach) can further improve the understanding of regulatory mechanisms altered in the cancer cell. A set of 254 metabolites (thereof 172 known metabolites) was semi-quantitatively determined by gas chromatography/liquid chromatography-mass spectrometry in matched malignant and non-malignant fresh-frozen prostatectomy samples from 95 prostate cancer patients. Transcription profiling data obtained from 15 PCA patients by means of Affymetrix U133 arrays was analyzed together with public expression data. Expression levels of selected proteins were determined by means of immunohistochemistry and tissue micro array technology in 41 matched frozen tissue samples. TMPRSS2-ERG translocation status was available from 30 patients. The association with clinicopathological variables and clinical outcome was tested. Transcription and metabolomics data were statistically analysed (ANOVA, Mann–Whitney U test) and significant differentially regulated metabolites/genes/proteins were selected. When comparing malignant tissue versus matched adjacent normal, 61% of the analyzed metabolites were significantly increased and 7% were significantly decreased after taking the false-discovery-rate into consideration. Differentially regulated metabolites/genes discrimination between malignant and non-malignant tissues was used for network analysis. Enriched pathways which are involved in PCA progression or recurrence such as carbohydrate and fatty acid metabolism were identified. The role of fatty acid metabolism in PCA was analyzed in more detail. Several fatty acids such as cerebronic acid, 2-hydroxybehenic acid, tricosanoic acid showed higher concentrations in malignant than in non-malignant tissues. This finding is in concordance to the observed higher mRNA and protein expression level of fatty acid synthase (FASN) in PCA. In contrast to normal prostate tissue, where protein expression level of FASN was correlated to the level of measured lipids we found in malignant tissues a deregulation of the corresponding pathway. The polyamine metabolism showed a marked pattern in malignant tissue compared to adjacent normal. Whereas putrescine and spermine were decreased, spermidine was increased. In the samples with an ERG translocation, several lipids were increased and changes in the level of amino acids observed. This metabolomics study of prostate cancer is the first to demonstrate metabolome changes in dependence of the TMPRSS2-ERG translocation status.

POSTER 041

NMR-based metabolomic profiling of intact prostate tissues for biomarker research

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Prostate cancer (PCa) is the second leading cause of cancer related deaths in men. Current diagnostic methods are unreliable at discriminating indolent from high-risk tumors. Therefore, there is an urgent need for i) identifying molecular markers suitable for early diagnosis and prognosis of prostate tumors, and ii) developing biomarker based treatment strategies of PCa. The TMPRSS2-ERG fusion gene is found exclusively in prostate cancer patients and is detected in about 50 % of all cases. TMPRSS2-ERG has been associated with aggressive cancer, reduced disease free survival and higher rate of prostate cancer specific death. We report a workflow that uses histologic and comprehensive metabolomics analysis on a single prostate tissue enabling the measurement of metabolites by 1H HR MAS NMR on the intact tissue (also used for histologic analysis) and 1H/31PNMR of tissue extracts and, subsequently, the direct analysis of the same samples by using LC/MS with no need for solvent exchange. We analyzed metabolomics profile of prostate cancer in relation to non-malignant prostate tissue, but also with respect to the presence of the fusion gene. In this study, we have proven the possibility to differentiate prostate cancer from adjacent non-malignant tissue with high sensitivity and specificity based on metabolic profiling. Tumor samples were shown to contain higher levels of glutathione, glutamate, aspartate, and the choline- containing metabolites, in addition to lower levels of polyamines and citrate compared to non-malignant tissue. We present the effort to identify metabolomic markers associated with ERG Rearrangement-Positive (ERG+) and -Negative (ERG-) prostate cancer tissues, which could help to understand the mechanism by which the TMPRSS2-ERG fusion may promote progression of prostate cancer. We show that prostate cancer exhibits distinct metabolomics patterns associated with ERG status. The detected changes included trends of increasing levels of myo-inositol and decreasing levels of phenylalanine, tyrosine and tryptophan in ERG+ compared to ERG- prostate tissue. Our observation support the current view that ERG+ prostate cancer and ERG- prostate cancer should be considered as different diseases requiring possibly different treatment strategies. Because of the fundamental differences in the nature of NMR and LC-MS measurements, the results obtained using each platform contributed to a complementary understanding of the complex metabolic alterations in prostate cancer and enabled evaluation of biomarkers related to occurrence, amount and aggressiveness of prostate cancer. Comprehensive metabolomics analysis of prostate cancer and adjacent non-malignant prostate tissue, as well as of genetically different prostate cancer subtypes.

POSTER 042

Plasma metabolites in pancreatic cancer–associated diabetes – as candidate early-detection biomarkers

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Pancreatic cancer (PDAC) is a devastating malignant disease with low level of early detection. PDAC has been reported with high association of development of glucose intolerance and dysregulation. Applying this clinical information as well as the previous reports of strong potential for the application of metabolomics approach in development of early detection biomarkers for PDAC, we have pursued further characterization of plasma metabolites for discriminating PDAC cases among diabetic patients as a means to delineate subset of patients who may benefit from a screening protocol utilizing currently available clinical imaging tests. IRB-approved prospectively collected database and corresponding plasma from UC Davis Pancreas Registry was searched for appropriate cases: PDAC patients with recent-onset diabetes (≤3yrs prior to the index PDAC diagnosis) (Grp 1) and non-PDAC patients with long history of diabetes mellitus (>3 yrs) (Grp 2). The plasma was processed and non-targeted GC/MS and targeted LC/MS were performed to assay for identifiable plasma metabolites. The data was processed and the peak intensity levels were normalized, scaled and identifiable metabolites were analyzed via

metabolomics specific multivariate & bootstrap methods as well as ROC evaluation (MetaboAnalyst3.0) for visualization, pathway analysis, and biomarker development. We have identified 36 patients in Grp 1 and 22 patients for Grp 2. The metabolite specific multivariate and ROC analyses revealed specific features such as elaidic acid, uric acid, 2,3-propanediol, PGF2alpha, arachidonic acid, docosahexanoic acid, 5-oxo-ETE, lysine, LysoPC(18:2), 9(10)-EpOME, LysoPC(16:0), sphingosine-1-phosphate and others as discriminators for recent-onset diabetic patients with pancreatic cancer. Such set yielded AUC of 0.937 with 20 features [CI=0.822-1] with random forest method. Pathway analysis revealed metabolites involved in aminoacyl-tRNA biosynthesis, arginine/proline, alanine/aspartate/glutamine, biotin, linoleic acid, and sphingolipid metabolisms among others were implicated. Our analysis resulted in a set of candidate plasma metabolite biomarkers for discriminating new-onset diabetic patients with developing pancreatic cancer.

POSTER 043

Comprehensive LC-MS and GC-MS Micrometabolomics of individual plasma and brain tissue samples

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In recent years the development of new methods has seen metabolomics progress from a novel analytical technique towards a mainstay of the biological toolbox. However before this transformation is completed a number of challenges remain including maximizing coverage of the metabolome. A number of studies have done this by analyzing samples on multiple platforms, and whilst this increases coverage it also increases the amount of sample required. As a result of this we aimed to develop a micrometabolomics method using a single split phase in-vial dual extraction from small sample volumes, subsequently analyzed using a combination of 6 LC-MS and GC-MS methods including hydrophilic liquid interaction chromatography, reversed phase and GC-MS analysis of aqueous and non-aqueous phases. Samples were initially analyzed by LC-MS with the aqueous phase separated using hydrophilic liquid interaction chromatography (Merck Sequant Zic-HILIC) and the non-aqueous phase separated using reversed phase chromatography (Agilent Poroshell 120 EC-C8). After LC-MS analysis the remaining extract was split and the aqueous and non-aqueous phases were independently derivatized using N,O-Bis(trimethylsilyl)trifluoroacetamide and Trimethylchlorosilane. Samples were subsequently analysed on a BP5MS column (30m length, 0.25mm diameter and 0.25mm thickness), using a helium carrier gas. All data was processed using XCMS performed in the open source software package R using appropriate parameters, with processed data subsequently analyzed in SIMCA 13.0.3 (Umetrics, Umeå, Sweden) using principle component analysis (PCA) and orthogonal projection to latent structures (OPLS). In this study we developed two in-vial dual extraction methods requiring just 3mg of brain tissue and 20µl of plasma, from which a total of 20,000 and 18,000 (signal to noise ratio >5) metabolite features were measured in brain tissue and plasma respectively. In the aqueous phase approximately 10,000 metabolite features were measured in both brain tissue and plasma of which over 200 features were successfully annotated with metabolites including amino acids, sugars and nucleotide bases. In the non-aqueous phase approximately 10,000 metabolite features were measured in brain tissue with slightly less (approximately 8500) measured in plasma, of these features over 400 were successfully annotated with metabolites including phosphatidylcholines, sphingolipids, glycerides and fatty acids. To validate the performance of the method developed it was applied to a small pilot experiment of 40 plasma samples (20 vs. 20, control vs. Alzheimer's disease). The method was shown to be capable of discriminating healthy controls from Alzheimer's disease patients, with all data combined showing the greatest class separation (R2Y = 0.891, Q2 = 0.745, CV-ANOVA = 4.2×10-9). From this data we were able to identify a number of previously identified AD biomarkers including glutamate and uric acid as being associated with disease pathology, as well as identifying potential new AD biomarkers including iminobutyl-ornithine (p = 4.01×10-8) and methylcaffeine (p = 3.60×10-4). The ability of this method to measure 20,000 metabolite features from 20µl of plasma and 3mg of brain tissue.

POSTER 044

Comprehensive metabolomic and lipidomic profiling of kidney tissue using liquid-chromatography quadrupole time-of-flight mass spectrometry

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Metabolomics, the large-scale analysis of small molecules and lipids, is rapidly establishing itself as a powerful tool for comprehensive characterization of human tissue samples. The enormous structural variety of metabolite classes present in different tissue types requires the continuous development and refinement of tissue-specific sample preparation procedures. Here we describe a two-step extraction protocol for the consecutive recovery of polar metabolites and non-polar lipids from human kidney samples. Metabolic extracts were analyzed by liquid-chromatography time-of-flight mass spectrometry (LC-QTOF-MS) in positive and negative ion mode to maximize metabolome coverage. The presented approach provides a first basis for comprehensive metabolomics studies in kidney tissue and thus offers great potential for the discovery of novel, diagnostic and prognostic biomarkers of renal diseases. Metabolites and lipids were recovered from porcine and human kidney tissue samples (10-45 mg) by a two-step extraction protocol. Tissue homogenization and extraction of polar metabolites was performed in methanol/water by a bead-beating approach. After centrifugation, lipids were recovered by consecutive extraction of the pellet with methanol/methyl tert-butyl ether. Metabolites recovered in aqueous extracts were separated by hydrophilic liquid interaction (HILIC) chromatography whereas compounds recovered in organic extracts were separated by reversed phase chromatography (RP). Mass spectrometric analysis (ESI+ and ESI-) was performed by QTOF-MS. Pooled QC samples were analyzed in parallel and used for column conditioning and to assess the analytical performance. MS/MS spectra were acquired in QC samples to support the identification of metabolites. The development of an analytical approach for the consecutive recovery of polar and apolar metabolites and lipids from tissue samples requires initial optimization of the homogenization procedure. The impact of lysing matrix type, composition of homogenization solvent and solvent-to-tissue ratio on the homogenization process was first evaluated on a visual basis using porcine kidney. Complete homogenization of kidney tissue was achieved in a mixture of cold methanol:water within 1-3 repeated cycles (20 sec. and 6.5m/s). Reproducibility of the two-step extraction protocol was assessed by LC-QTOF-MS-based analysis of aqueous and organic extracts from a set of three independently prepared porcine kidney samples. Pooled QC samples showed minimal variations in the total ion chromatogram substantiating robust analytical performance. More than 1000 metabolic features could be detected in all replicates with coefficients of variations (CVs) less than 20% in each extract (organic and aqueous) and each ionization mode. Carryover was determined for HILIC and RP by the analysis of selected metabolites (e.g. amino acids, carnitine and triglycerides). The selected compounds exhibited only weak signals (less than 0.5%) in blank samples that were acquired after tissue extracts indicating neglectable carryover of the analytical approach. To demonstrate the applicability of the two-step extraction protocol for comprehensive metabolomic and lipidomic analysis in clinically relevant tissue samples, the approach was applied to kidney tissue derived from a human biobank. As observed for porcine kidney, more than 1000 metabolic features could be reproducibly detected in ESI(+/-)-mode in the organic and aqueous extracts indicating comparable metabolic profiles of human and porcine tissue. On the basis of accurate mass and fragment spectra information a broad spectrum of kidney-derived metabolites including fatty acids, glycerophospholipids, sphingholipids, glycerolipids, acylcarnities, aminos acids, carbohydrates, nucleosides and organic acids was tentatively identified. A dedicated two-step extraction protocol for the combined analysis of small molecules and lipids in human kidney is provided.

POSTER 045

Improvements in accurate mass GC-MS based Metabolomics: A novel atmospheric pressure GC-APCI-Source increases quantitative and qualitative performance for metabolic profiling

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Gas chromatography coupled to mass spectrometry (GC-MS) is one of the most widely used analytical techniques in metabolomics. While electron ionization (EI) is the common analytical standard for GC-MS, atmospheric pressure chemical ionization (APCI) became more prominent in recent years. The soft APCI ionization preserves molecular information and opens the doors to the world of unidentified metabolites which could not yet be annotated due to missing library data. We will report the application of a novel GC-APCI design coupled with high resolution oTOF-MS to analyse derivatized metabolite extracts and reference standards. Compared with earlier results [1], an improved analytical performance resulted in a higher number of compounds which could be identified in human cancer cell extracts. Cell culture supernatants of pancreatic cancer cells (MiaPaCa-2 cells) and pure standards were methoximated and trimethylsilylated, followed by GC separation using a Rxi-5ms (Restek) column. The GC column was interfaced to an orthogonal TOF (impact HD Q-TOF-MS or micrOTOF ESI-TOF-MS, Bruker Daltonics) by a novel APCI source operated in positive ion mode. Data was acquired at m/z 50-1000 with a spectral rate of at least 3 spectra per second to cope with the GC separation speed. Spectra were externally calibrated with PFTBA as a calibrant, which was injected automatically into the APCI source prior to each GC/MS run. DataAnalysis 4.1 and ProfileAnalysis 2.1 were used for data evaluation (Bruker Daltonics). For APCI it is absolutely essential to maintain stable ionisation and temperature conditions for the water cluster ionisation process. The novel GC-APCI design thermally shields the ionisation volume and thus enables lower chemical background, increased analytical sensitivity and maintained GC separation resolution. Upon comparing previous APCI-I [1] and novel APCI-II ion sources, GC-APCI-II-TOFMS analysis resulted in improved peak shapes and much better peak area reproducibility for fatty acid methyl esters (FAMEs). Furthermore, overall decreased lower limits of quantification in the sub-micromolar range were found for twenty metabolites due to reduced background in the ion source. Meanwhile, the analytical linear working range was either maintained or slightly increased. The improved analytical performance enabled to approximately double the number of extracted peaks with signal-to-noise ratios >20 in cell culture supernatant samples of pancreatic cancer cells. Injecting PFTBA automatically into the source before each GC/MS run, lead to low ppm mass deviations for the standard compounds analyzed in this study. The higher number of peaks extracted and improved mass accuracy resulted in 36% more compounds which could be identified compared to the previous setup. In summary, APCI-II has a number of notable improvements over APCI-I and holds great promise for further studies in metabolomics. A novel GC atmospheric pressure chemical ionization (APCI) source for improved quantitative and qualitative data in metabolic profiling by GC/MS.

POSTER 046

Influence of Flow Cytometry Sorting on the Metabolome of Mouse Peritoneal Macrophages

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Metabolomics studies of cell lines have a growing research interest; however, little is known about the impact of cell manipulation on abundance and behavior of certain groups of metabolites. In particular, metabolite stability in cell lines that undergo fluorescence-activated cell sorting (FACS) is highly controversial. To address this issue, we designed a proof-of-concept experiment to assess FACS-induced changes in the metabolite composition of mouse macrophage cells by LC-MS. C57BL/6 mice were injected with thioglycollate medium into peritoneal cavity, and macrophages were collected after 72h. One half of the cellular pellet was snaPoster frozen immediately after isolation to be used as a control, whilst CD45+ cells from the second half were subjected to FACS. The metabolites were extracted from the cellular pellets with i) MTBE:MetOH; and ii) MetOH:H2O (Naz et al., 2013), and the resulting supernatants were pooled and analyzed in LC-ESI-QTOF-MS (1290 infinity coupled to 6550 iFunnel, Agilent) (Rojo et al., 2015). They were injected five analytical replicates. QC samples were prepared by pooling equal volumes of the two metabolite extracts and injected in between blocks of three samples until the end of the run to ensure analytical reproducibility. After feature extraction (Mass Hunter Qualitative Analysis software, B.05.00, Agilent) and alignment (MassProfiler Professional software, B.12.01, Agilent), the data were processed according to the Quality Assurance Plus procedures developed by (Godzien et al., 2014). The PCA score plot showed adequate QC clustering and therefore ensured the biological differences between groups (control and sorted). MATLAB scripts based on Mann-Whitney U test built at CEMBIO were used for the statistical analysis. A non-normal distribution of the features was assumed as it could not be tested due to the limiting number of replicates. The p value post-correction was performed using the Benjamini-Hochberg method. Statistically significant changes ($p < 0.05$) were found for 456 and 415 features detected in the positive and negative mode, respectively (33% of the 2600 features found). The mass features were putatively identified using CEU Mass Mediator, a search engine that interrogates METLIN, KEGG and LIPID MAPS online databases. A total of 749 mass features were attributed to compound identities, out of which 293 were significantly changing due to FACS process. The majority of the metabolites found altered belong to three chemical classes: glycerophosphocholines, glycerophosphoethanolamines and glycerophosphoserines, which constitute main components of cell membranes. On the other hand, many metabolites implicated in a variety of cellular metabolic processes, such as fatty acids, fatty esters, bile acids, and isoprenoid compounds failed to show statistically significant changes. Our results evidence that FACS may have a significant impact on the stability of cellular membrane components.

POSTER 047

Rapid and robust profiling of phospholipids by HILIC-Ion trap FTMS and its application to EAE rat model of multiple sclerosis

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Phospholipids, a major part of all cell membranes, play very important roles in organisms, some of which were identified as potential biomarkers associated with different diseases. Herein we developed a rapid and robust method for the profiling of phospholipids in plasma and liver based on HILIC-Ion trap FTMS. Experimental autoimmune encephalomyelitis (EAE) is the most common animal model used to study some aspects of multiple sclerosis (MScl). In a previous investigation we have reported changes in CSF metabolites related to the development of acute EAE in Lewis rats. As an addition to the previous study, we applied the developed method to investigate potential changes in the plasma and liver lipidome that may be associated with the development of EAE. With the use of a diol column, an acetonitrile-H2O-formic acid-ammonium format solvent system and a binary gradient, 9 phospholipids classes were profiled by LC/Ion trap FTMS in 9 minutes. A total of 186 compounds were identified based on retention time, accurate mass and characteristic fragmentation in negative ionization mode. The method was fully

validated with plasma and liver tissue samples. The performance characteristics, such as linearity (plasma, R > 0.992; liver, R > 0.991), repeatability (plasma, RSD < 6%; liver, RSD < 4%), intermediate precision (plasma, RSD < 6% ; liver, < 4%), recoveries (plasma, 90% ~ 123%; liver, 90% ~ 130%), LOD (0.0026 µg/mL) and LOQ (0.017 ~ 0.103 µg/mL plasma; 0.030 ~ 0.150 µg/mg dried liver), were satisfactory. The method was successfully used to profile the phosphor-lipidome of EAE rats. Both uPoster and down-regulation of specific lipids was observed in various stages of the EAE progression. However, an in-depth biological explanation of all variations is difficult and a causal relation is not yet apparent. In conclusion, several significant changes in plasma lipid levels were demonstrated in acute EAE in rat. Due to complex nature of EAE and possible interference of secondary effects, like weight loss, observed changes are difficult to interpret. On the other hand, these results show clearly that profiling of complex diseases requires comprehensive multi-compartment metabolomics approaches. A rapid and robust method for profiling phospholipids was developed based on HILIC-IT-FTMS as a new platform for metabolomics investigation.

POSTER 048

A Novel MSMSALL workflow for Unbiased Lipidomics Discovery for Serum Biomarkers

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Lipidomics discovery workflows are often conducted using the “shotgun lipidomics” approach, with multiple precursor-ion and neutral-loss scans (MPIS) performed on a triple quadrupole instrument via infusion of lipid extracts. Alternatively, lipid profiling can be performed via LC separation with an information-dependent acquisition strategy (IDA), wherein lipid species are separated chromatographically and detected by MS. These traditional approaches have limitations in terms of coverage as well as quantitative rigor, and it is a challenge to analyze the complete lipidome. In this study, a novel high-resolution-accurate-mass (HRAM) based MSMSALL workflow was established and used to examine serum lipids in diseased and control subjects. Using this quantitative data-independent approach, lipid profiles were acquired and potential biomarkers for disease diagnoses were identified. Serum was extracted using a modified Folch method. The organic phase was concentrated and analyzed with MSMSALL (fragment ion scans for all precursors) acquisition in both positive and negative ionization modes. Serum extracts were introduced to the MS with automated flow injection. Injected samples were continuously infused with a flow rate at 7 µL/min of dichloromethane/methanol (1/1 with 5mM ammonium acetate). 100 µL of sample provided more than 10 minutes MS acquisition time window. The TripleTOF® 6600 was operated in MSMSALL mode where all MS1 precursors were captured with a TOF full scan and the TOF MS2 fragment ion spectra scans were collected for each precursor from 200 to 2000 amu with unit isolation width. The preliminary study used serum from healthy controls (n=5) and diseased subjects (n=5). Lipid extracts were analyzed on the TripleTOF® 6600 using MSMSALL acquisition in both positive and negative ion modes. MSMSALL data were processed with LipidView® software in which lipid features were identified, and the lipid molecular species profiles were established for each sample. The number of features that were different between the two groups was close to 1,500. The established lipid profiles of the healthy control and diseased subjects were then processed by MarkerView® Software. Potential biomarkers were identified by principal component analysis (PCA) from diseased subjects with either elevated or suppressed concentrations compared to control samples. An automated, untargeted MSMSALL lipidomics workflow and its application to biomarker discovery is presented.

POSTER 049

Processing of a Complex Lipid Dataset for the NIST Inter-laboratory Comparison Exercise for Lipidomics Measurements in Human Serum and Plasma

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Lipidomics is a new field of study crucial for understanding cellular physiology and pathology. The application of lipidomic profiling to disease phenotype analysis is a rapidly growing aspect of translational medical research. Identification of lipids by untargeted lipidomics requires sophisticated software with an extensive lipid database. In addition, the mass spectrometer employed must be capable of separating many overlapping isobaric and isomeric lipid ions. We present here the details and challenges of the data processing of the NIST plasma and serum extracts using the latest version of LipidSearch software. New algorithms were introduced specifically to reduce false positives and to help automate the data review. Sample preparation and analysis of lipid extracts obtained from NIST human plasma (SRM 1950) and serum (SRM 2378-1, 2378-2 and 2378-3) are described separately. Human plasma and serum lipid extracts were separated via a C30, 1.9µm column prototype column using a Dionex 3000 RSLC chromatograph and LC-MS-MS was performed using the Thermo ScientificTM Q ExactiveTM HF Orbitrap mass spectrometer. The datasets from three separate extractions of the NIST samples were then processed using LipidSearch 4.1 software. This software contains new features such as peak quality, signal-to-noise and alignment algorithms designed to accurately identify and merge annotated peaks and improve data filtering tools for reviewing the lipid identification and relative quantification results. LipidSearch software (Thermo Scientific) was used for lipid identification through a database search of the accurate masses of precursors and the fragment ions predicted for each potential adduct form of the lipids in the database (> 1,500,000 entries). The acquired LC/MS-MS spectra were searched against the predicted fragment ions of all of the potential lipid species within ±5 ppm of the precursor ion mass to charge. Each lipid identification is ranked by mass tolerance, match to the theoretical fragmentation and the fraction of total MS-MS intensity for the predicted fragment ions. The number of lipid species identified in each different experiment were assessed at the sum composition (MS) and isomer (MS-MS) levels. For each LC-dd-MS2 run potential lipid species were identified using the predicted MS-MS fragments for the molecular species observed in the positive or negative ion mode. For positive ions, the unfiltered data gave 600-800 sum composition species and 900-1500 MS2 spectra representing possible isomeric species. The data for each run were aligned within a chromatographic time window and positive and negative ion data were merged into a results table. The alignment algorithm provides annotations that combine the positive and negative ion identification into the merged results. This approach provides lipid annotation that reflects the appropriate level of MS2 fragment ions from the complete dataset giving higher confidence in lipid identifications. The results were then filtered by main adduct ion, match score, ID quality, signal-to-noise, peak area and relative standard deviation; manual integration was performed if necessary prior to estimating concentration relative to an internal standard for each lipid class. These results demonstrate that in a 60 min LC-MS run that it is possible to separate and identify over a thousand isomeric lipid species from human plasma using a C30 UHPLC column. Identification and relative quantification of lipids in human plasma/serum using high resolution LC-MS-MS data and new algorithms in LipidSearch software.

POSTER 050

Large Scale Lipid Profiling of a Human Serum Lipidome Using a High Resolution Accurate Mass LC/MS/MS Approach

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HPLC-MS platforms are increasingly used for large scale lipid profiling experiments aimed to discover new biomarkers for early disease diagnose. It is critical that the adapted LC/MS platform offers the capability to separate and identify the isobars and isomers from the biological lipid extracts. C30 HPLC columns with small particle size uniquely offer high shape selectivity for separation of structurally related isomers and provide improved lipid isomer separation efficiency compared to C18 columns. Here we report that thousands of lipid molecules from human serum sample were simultaneously identified and quantified using a new C30 column and a quadrupole Orbitrap HR/AM MS platform. Human serum samples provided by NIST were collected from three types of donors with different controlled diets. The profiling of total lipid extracts from the human serums was performed by UHPLC-MS using a C30 prototype column (2.1x250mm, 1.9µm) and the Thermo ScientificTM Q ExactiveTM HF mass spectrometer. MS and MS/MS data were obtained at 120,000 and 30,000 mass resolving power, respectively. Each serum sample was run in triplicate with positive mode and negative mode ionization. The HPLC gradient was 60:40 acetonitrile/water to 90:10 IPA/acetonitrile (0.1% formic acid, 10mM ammonium formate) in 58 min. The flow rate was 200µL/min. Thermo Scientific™ LipidSearch™ version 4.1 software was used for lipid identification and quantitation. The total lipids were extracted from the human serum samples by using organic solvents of chloroform, methanol and water. Multiple internal lipid standards which represent typical lipid classes were spiked into the serum samples prior to the lipid extraction. One of the unique feature of C30 column compared to the C18 column is the higher shape selectivity to allow better separation of hydrophobic, long-chain, structural isomers of the lipid extracts. Many lipid isomers which were separated on the C30 column were not separated on C18 columns, such as PC (18:1/21:5), TG (14:0/16:0/16:1) and TG 16:0/16:1/17:1). With our QC sample (bovine liver lipid extracts from Avainti), overall 15% more lipid species were identified from 500 ng lipid extract using the new C30 column compared to a C18 column (Supelco, Ascentis C18, 2.1x150mm, 2.7µm) using a 58 min gradient. The combination of increased lipid isomer separation offered by the C30 column and faster scan speed and high resolving power offered by the Q Exactive HF MS enabled very high lipid identification coverage to be achieved using the high resolution accurate full scan MS data and MS/MS data collected from the total lipid extract of the human serum samples. With the preliminary data processing results, more than 1300 lipid molecules were identified with high confidence by the LipidSearch software. The data processing progress is ongoing for quantitative analysis. Increased lipid identification coverage and lipid profiling throughput by using a new C30 column with a Q Exactive HF MS.

POSTER 051

Rapid Phospholipid Characterization Using a Novel Intelligent Workflow on a Tribrid Orbitrap Mass Spectrometer

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High energy HCD/MS/MS is widely used for the characterization of lipid extracts. Both positive and negative HCD MS/MS data are often required for fully characterization of individual molecular species, such as PCs. It takes extra instrument time to collect both positive and negative MS/MS data and the ionization efficiency in the negative mode is generally lower. In order to address this issue, we developed a novel workflow on a Orbitrap Fusion instrument, which uses HCD MS/MS for characterization of most lipid classes and combined HCD/CID MS/MS for characterization of PC class. Here we report that individual PC molecular species can be fully characterized with other lipids together within a single positive LC/MS/MS run using this new approach. Egg PC and human serum were used as test samples. All experiments were performed using a Thermo Scientific™ UltiMate™ 3000 RSLC pump connected with the Thermo Scientific™ Orbitrap Fusion™ mass spectrometer. The HPLC gradient was 60:40 Acetonitrile/Water to 90:10 IPA/Acetonitrile (0.1% formic acid, 10mM ammonium formate) in 24 min using a Thermo Scientific™ Accucore C18 column (2.1 x 150mm, 2.6µm) operated at 400µL/min and 45°C. An intelligent data dependent workflow was used, in which a full MS scan (120K) was followed by top15 HCD MS/MS (30K) and an additional CID MS/MS (30K) was triggered if the polar head group of PC fragment ion (184.0733) was detected from the HCD spectrum. Thermo Scientific™ LipidSearch™ software was

used for lipid identification. The Egg PC purchased from Avanti was used to test the initial concept of this workflow. The same amount of the Egg PC (500 ng on column) was analyzed with three different approaches, including 1) the intelligent workflow described above, 2) Top 15 MS/MS data dependent experiments on both positive mode and negative mode, respectively and 3) Top 10 MS/MS data dependent experiment using positive/negative online switching. The collected HR/AM MS and MS/MS data obtained using the three approaches were processed by LipidSearch software. LipidSearch software automatically combines the HCD and CID MS/MS data collected from the same precursor ion as a single MS/MS spectrum for the lipid identification and characterization. LipidSearch also allows aligning positive and negative ion data from multiple raw files for automatic lipid identification and characterization. There advanced features of Lipid Search software enabled confident lipid identification/characterization from all three approaches. In order to evaluate the productivity and sensitivity improvement for PC characterization of the intelligent workflow, the total number of identified sum composition and individual PC species, and the percentage of confidently characterized PCs at the individual molecular level observed were compared from each approach. More than one hundred PC lipids were identified and were able to be characterized using rich fragment ion information from the combined HCD and CID MS/MS data, or alignment of positive/negative MS/MS data. More low abundance PC species were fully characterized using the new workflow because of the higher ionization efficiency in the positive ion mode compared to negative ion. The intelligent workflow developed here was then applied to the analysis of total lipid extracts obtained from human serum samples for large scale lipid profiling with on-going experiments. Improved productivity and sensitivity for Phosphatidylcholine characterization using a novel intelligent hybrid dissociation workflow on an Orbitrap Fusion MS.

POSTER 052

Tissue Sample Preparation Optimization for Global Lipidomic Analysis

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Optimization of sample preparation is important to ensure there is minimal variation between samples. Untargeted metabolomics aims to be a global application but every step can introduce chemical bias. Two different animal tissue samples were used for optimization of buffer type and concentration during the homogenization step of tissue sample preparation, liver and muscle tissue. The samples were then subjected to organic extraction, either Folch or Matyash, to determine greatest lipid coverage. This experiment aimed to evaluate the bias involved in sample preparation for detection of as many metabolites as possible from homogenization, extraction and chromatography, onwards. Liver and muscle tissue were homogenized using 10 and 50 mM ammonium acetate, PBS and water, then extracted using the Folch method. Previously optimized LC-MS conditions used a Waters C18 column with Dionex UHPLC and Thermo Q-Exactive instrumentation. The samples were analyzed using polarity switching with pooled samples subjected to all ion fragmentation. Ideal buffer and concentration was defined as the combination that produced the least variation in signal for a wide variety of compounds. Following the buffer experiments, the extraction efficiencies of the Folch and Matyash methods were examined for comparison. Internal standards were spiked in before extraction and a different set of standards were spiked in after extraction. The highest ratios of before/after yielded the best extractions. In our previously conducted experiments, a comparison of the Folch extraction method versus the Matyash method was conducted using blood plasma. It was shown, in this case, that the Matyash method provided the best recovery across compound classes at almost 100%. Current metabolomics research includes tissue analysis, therefore this experiment was expanded to look at these methods on different types of tissue. The LC gradient was optimized for separation and throughput for the lipidomics application. LC columns from different vendors were compared on factors such as peak shape and peak capacity. It was determined that the Waters Acquity BEH C18 column was most suitable for our application. The LC gradient was also fully optimised to be held 20% solvent B at the start for 1 minute before gradually increasing to 98% B over 16 minutes. Gradient was held isocratic for 1 minute before returning to starting conditions over 1 minute and re-equilibrating for 4 minutes. In addition, polarity switching mode on the Q-

Exactive proved useful in decreasing the number of injections needed in a run whilst increasing the overall throughput. Despite the lower resolution of 35,000 from 70,000, the resolving power and accurate mass proved to be adequate for the lipidomic application. In a comparison of buffer types, the lowest variation occurred with the 10 mM ammonium acetate for liver and the 50 mM ammonium acetate for the muscle. This optimization of both the buffer type and concentration, enabled wide coverage of lipids present in the sample. Using this information, current work comparing the extraction efficiency of the Folch and Matyash methods on muscle and liver tissue with the above ideal buffers enables optimal extraction efficiency, recovery and reproducibility. The first comparison of the Folch and Matyash extraction methods in combination with homogenization buffers on muscle and liver tissue.

POSTER 053

Profiling the Pyridine Nucleotide Pool in Skeletal Muscle From Mice That Overexpress Nicotinamide Phosphoribosyltransferase (NAMPT)

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Nicotinamide phosphoribosyltransferase (NAMPT) catalyzes the formation of nicotinamide mononucleotide (NMN) which is used by NMN adenyltransferase (NMNAT) to produce NAD+. We previously showed that exercise training increased skeletal muscle NAMPT protein in humans and that NAMPT levels correlate with maximal aerobic capacity (VO2max) and mitochondrial content. Transgenic mice specifically overexpressing NAMPT in skeletal muscle (NamptTg) were created to test if the putative beneficial effect of elevated NAMPT was recapitulated in a mouse model. Herein, we assessed the impact of NAMPT overexpression in skeletal muscle on the levels of NMN, NAD+, NADH, NADP, and NAPDH. Our metabolite profiling was accomplished using refined tissue processing methods and optimized LC/MS/MS protocols that employed heavy-isotope labeled internal standards (NAD+, NADH and NMN). Tissues were harvested from pentobarbital-treated mice and flash frozen. Lyophilized and powdered tissues (~10 mg) were homogenized in 500 µL 0.5 M PCA (for NMN, NAD, and NADP) or 500 µL 50:50 0.1 M NaOH/MeOH (for NADH and NADPH). PCA extracts were neutralized with 1 M ammonium formate. Aliquots of homogenates were spiked with 18O2-NMN, NAD or NADH and passed through 3 kDa filter plates. Samples were analyzed by single reaction monitoring using a Dionex 3000 HPLC and Hypercarb column (3 µm, 50 x 2.1 mm) which was interfaced to the positive ESI source of a Thermo Scientific Quantiva triple quadrupole mass spectrometer. Calibration curves (0.025 to 200 µM) had R2 ≥ 0.99 and accuracies ± 20% of theoretical values. NamptTg mice express approximately 8-fold-fold more skeletal muscle NAMPT protein compared to wild type (WT) mice based on western blotting analysis. The whole gastrocnemius muscle powder prepared from the NamptTg mice displayed a 2.2-fold increase of NMN as compared to WT mice. This result revealed that the increased NAMPT expression in gastrocnemius muscle was accompanied by higher NAMPT enzymatic activity. There was a concomitant 1.7-fold increase of the NAD+ level in gastrocnemius muscle harvested from NamptTg mice compared to WT mice. It is important to note that the levels of NADH, NADP and NADPH were not altered in gastrocnemius muscle powder prepared from NamptTg mice. However, the crucial NAD+/NADH ratio was 2.1 and 3.5 in the gastrocnemius muscle from WT and NamptTg mice, respectively. The increased abundance of NAD+ in the skeletal muscle from the NamptTg mouse might promote enzyme reactions involving sertuins (deacetylation and other deacylase reactions) and ARTs/PARPs (ADPOSTER ribosylation of protein acceptors) that use NAD+ as a co-substrate. On the other hand, the altered NAD+/NADH ratio might impact the activity of the numerous oxidoreductases that participate in a host of seminal catabolic pathways. It is revealing that the NADP(H) pool in the gastrocnemius muscle was not impacted by the increased NAD+ level mediated by NAMPT-overexpression. Knowledge about the pyridine nucleotide pool in skeletal muscle derived by using our optimized sample preparation and LC-MS/MS method is vital for our ongoing attempts to establish the mechanism for the altered phenotype displayed by the NamptTg mouse. Profiling of the pyridine nucleotide pools in skeletal muscle from NamptTg mice using optimized tissue processing and LC/MS/MS methods.

POSTER 055

Supercritical fluid chromatography-mass spectrometry methods for lipidomics profiling

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Long chromatography methods are often used for metabolomics and lipidomics profiling experiments due to the highly complex nature of biological samples and matrices, often resulting in extremely low throughput for 'omics experiments. When combined with the large numbers of samples needed to ensure good statistics, this work is time consuming and expensive. The use of supercriticial fluid chromatography (SFC) instead of liquid chromatography offers significantly shorter runs while preserving or even improving chromatographic separation for certain classes of lipids. Little work exists with annotated features, as such there is not widespread understanding of lipid and lipid class elution patterns by SFC. Better understanding of lipid class behavior in different SFC separations, scientists can better select optimum chromatography. An SFC coupled to a QTOF mass spectrometer, where the full flow from the SFC was sent to the ionization source, was used for this work. Two main chromatographic approaches were investigated, one using a C18 column and the other a CN column. A number of biological samples were studied, including the chloroform fraction of SRM 1950, a NIST/NIH standard reference of human plasma, and bovine and human liver. Cooking oils were analyzed primarily for their glycero- and phospholipid content. A number of standard lipids representing a variety of lipid classes were also analyzed in order to better characterize elution profiles of lipid classes in these methods. SFC-MS was found to be an excellent platform for lipidomics profiling experiments. The chromatographic reproducibility was more than sufficient, and the approach had several unique strengths, the most obvious of which was speed. Triglycerides (TG) are a lipid class where SFC is particularly advantageous. A mixture of five TG standards (C:8-C:16) were analyzed in liver matrix by both reverse-phase C18 LC and C18 SFC with a CO2-MeOH gradient. Using a 38 minute H2O-IPA LC gradient, the five TG standards elute over a 35 minute period. However with a 13 minute SFC method, the same five TG standards elute completely resolved within 5 minutes. Using the same two LC and SFC methods, bovine liver samples were annotated by searching against the METLIN database. The majority of TGs eluted at high IPA in a 2 minute window by LC. With the 13 minute SFC method, annotated TGs elute over a 10 minute window – an improvement in both chromatographic resolution and speed. SFC with a C18 column shows excellent separation of TGs based on chain length and degrees of saturation, giving baseline separation of TGs that differ by a single double bond. Trends for other lipid classes, as determined through SFC-MS analysis of chemical standards and annotated biological samples, will also be discussed. Evaluation of SFC-MS methods, using chemical standards and annotated features to illustrate lipid class behavior for use in lipidomics profiling

POSTER 058

A Novel Lipid Screening Platform Allowing a Complete Solution for Lipidomics Research

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A major challenge in lipid analysis is the many isobaric and near isobaric interferences present in highly complex samples that confounds identification and accurate quantitation. This problem, coupled with complicated sample preparation techniques and data analysis, highlights the need for a complete solution that addresses these difficulties and provides a simplified method for analysis. A novel lipidomics platform was developed that includes simplified sample preparation, automated methods, and streamlined data processing techniques that enables facile, quantitative lipid analysis. Herein, serum samples were analyzed quantitatively using a unique internal standard labeling protocol, a novel selectivity tool (differential mobility spectrometry; DMS) and novel lipid data analysis software. Applying the kit for

simplified sample extraction and preparation, a serum matrix was used following the protocols provided. An LC-DMS-QTRAP® System (SCIEX) was used for targeted profiling of hundreds of lipid species from 10 different lipid classes allowing for comprehensive coverage. This system allows for: 1. quantitative results for each lipid class as a sum of individual species; 2. mole percent composition was obtained computationally from lipid molecular species data; and 3. accurate lipid species compositions. The data was compared with historical data generated by alternative methods. Samples were quantitated using software accompanying the full solution which incorporates the novel labeled internal standards available as a kit, developed for this platform (Avanti Lipids). Quantitative lipid species measurements were obtained from the following complex lipid classes: diacylglycerols (DAGs), triacylglycerols (TAGs), phosphatidylcholines (PCs), phosphatidylethanolamines (Pes), sphingomyelins (SMs), lysophosphatidylcholines (LPCs) and lysophosphatidylethanolamines (LPEs), free fatty acids (FFAs), cholesterol esters (CEs) and ceramides (CERs). Covering these classes required a multi-injection approach but data can be collected in short, fast graidnts up 5 inutes run time per injection (for three injections). The three injections classes covered TAGs and SMs, CEs and DAGs and FFAs, PE/PCs and their lyso components (LPC/LPEs). A novel, quantitative lipid screening platform allowing a complete solution for lipidomics research.

POSTER 059

A HILIC-TOF method for untargeted lipidomics allowing for simultaneous quantification of multiple lipid species.

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Lipids play a key role as inflammatory mediators. Hence, analysis of lipid patterns can help to elucidate the activation of inflammatory pathways in disease. Currently, liquid chromatography coupled to mass spectrometry (LC-MS) is the method of choice for lipidomics. The reverse phase columns or direct infusion techniques typically used lack separation of isobaric lipids or applies inadequate non co-eluting internal standards (IS). Additionally, lipid identification by untargeted lipidomics requires comprehensive databases including retention times and fragmentation patterns. The fragmentation spectra needed are often not available because the analytes of interest are not known in advance. A method which can overcome these problems is hydrophilic liquid interaction chromatography (HILIC), which allows simultaneous identification and quantification of the lipids. Lipids were extracted from serum using an adapted Bligh&Dyer method. The samples were separated by ultra-performance LC (UPLC) equipped with a HILIC column. Using a high-resolution and accurate time-of-flight MS (TOF-MS) for detection, the method was not limited by dwell-time and hence feasible for quantification as well as for untargeted lipidomics. Validation of the quantification method for lysophosphatidylcholine species (LPC) was performed using LPC 19:0 as IS and 4 calibration standards (LPC 12:0, LPC 17:0, LPC 16:0 and LPC 18:1). This method was applied for investigation of 31 patients with community acquired pneumonia (CAP). Sera were obtained at 3 time points from each patient during the acute phase of infection and at >60 days after recovery. By applying a HILIC-TOF methodology to serum samples, it was possible to separate lipid classes while co-eluting the molecular lipid species which simplified the identification of lipids according to their highly accurate mass. Due to co-elution it was possible to use unlabeled standards and an IS not detectable in human serum. For quantification of LPC species, the method was calibrated between 0.5 µM and 300 µM. The 4 LPC standards showed high intraday accuracies ranging between 81-113% for high (50 µM; N = 5) and low (0.5 µM; N = 6) LPC levels spiked to serum, or water respectively, with RSD values < 10% (RSDs < 15% for interday comparisons). The lipids extracted were found to be stable for at least 6 days after sample preparation when stored at 4 °C. Finally, this method

was applied for untargeted lipidomics of serum from patients with CAP and concentrations of LPC species were found to be significantly decreased during acute disease. Using a within batch calibration curve based on 4 LPC standards, 15 LPC species were found to be above the lower limit of quantification (0.5 µM). All these 15 LPCs were significantly decreased in the acute phase of pneumonia confirming the results of the untargeted approach. For the first time, a detailed concentration profile of LPC species in patients with pneumonia could be analyzed. These results may help to understand the inflammatory processes involved in CAP. This method is applicable for untargeted fingerprinting with straightforward hit identification and includes a possibility for simultaneous quantification of lipids.

POSTER 061

Enhancing the quantification of amino acids by 1H-NMR spectroscopy, considering their interaction with human serum albumin

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Recent clinical trials have shown the important role that some amino acids found in serum (phenylalanine, valine, isoleucine, leucine and tyrosine) could play in the early detection of diabetes type 2 and cardiovascular events. High-throughput 1H-NMR profiling is a low cost and non-destructive sampling method widely used in metabolomics. However, quantitative concentration analysis of low molecular weight metabolites (LMWMs) may be obscured by large errors caused by interactions between LMWMs and proteins, (especially Human Serum Albumin (HSA)), making the tightly bounded part of LMWMs invisible in 1H-NMR. Strategies for improving the quantification of LMWMs include the study of spin-spin (T2) relaxation times and the use of chemical compounds that compete with LMWMS for HSA binding, promoting the release of LMWMs. Globulin and fatty acid free HSA, L-Phenylalanine, L-Valine, L-Isoleucine, L-Leucine, L-Tyrosine and TMSPOSTER d4 from Sigma Aldrich were prepared in stocks solutions of sodium phosphate buffer (pH 7.4, 0.05 M) containing 10% D2O. A series of titrations of amino acids into HSA solution were prepared fixing HSA (0.3 mM and 0.03 mM) and ratios LMWM-HSA ranging from 0.25 to 500. Finally, 10 mM of TMSPOSTER d4 was added to HSA 0.3 mM series. CPMGpresat was used with T2-filters ranging from 0 to 2.5 s. Multivariate Curve Resolution (MCR) was used to extract pure spectra and concentrations from individual components. Concentration was calculated from the 0 seconds filter and T2 was obtained by fitting the concentration decay for the spectral area of interest. T2 and concentration of each LMWM were monitored versus the ratio LMWMs:HSA in the titration series. Observed T2 represents the ratio between the free and weakly-bound portions of LMWM whereas measured concentration curves give the “visible” part (free + weakly-bound portions) in the 1H-NMR domain. Strongly-bound part (NMR “invisible”) is derived from the measured concentration as the added LMWM quantity is known. Titrations curves reveal the binding patterns for each of the LMWMs under study and show a high degree of correlation between strong and weak interactions. Whilst for large ratios of LMWM:HSA the saturation state is reached and T2 approximates the free value and its recovered concentration approaches 100%, for the commonly reported human concentrations, aggregation curves are flat and far from the saturation levels. In this region, less than 50% of signal is recovered from the total LMWM added. Concerning the five amino acids under study, phenylalanine and valine show the strongest and weakest aggregation, being possible to recover 20% and 40% of the total concentration, respectively. In order to promote disaggregation of LMWMs, two strategies have been implemented: a) Dilution of the samples: this enables to increase the “visible” portion of LMWM, at the expense of lower S/N ratio or longer acquisition times. b) Binding competition: TMSPOSTER d4 was used to displace phenylalanine and increase its recovered concentration from the 20% to 70% of the total added. The latter strategy preserves the S/N ratio and ensures compatibility with high-throughput 1H-NMR profiling. Further studies are needed to better improve the targeted release of each LMWM of interest from HSA. Improve LMWMs quantification in high-throughput 1H-NMR profiling of serum by increasing their “visibility” through adding high HSA affinity compounds.

POSTER 062

Long-term stability of plasma metabolites stored at -80°C

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Metabolomics has proven as a versatile tool in many epidemiological studies. However, even small perturbations in the pre-analytical procedure can alter the metabolite profile and bias the statistical analyses of the data. While blood sampling and handling can be controlled, alterations of plasma metabolite concentrations during storage cannot be avoided completely. The majority of the existing pre-analytical studies were focused on short-term stability and effects of repeated freeze-thaw cycles. When analysing retrospective samples, for instance in longitudinal studies, storage times of several years can occur. In our work, we demonstrate the time-dependent course of the metabolite concentrations of a pooled reference plasma sample that was repeatedly measured with the Biocrates AbsoluteIDQTM p180 kit over a time period of four years. A large amount of pooled human plasma sample was aliquoted and immediately stored at -80 °C. Over a time period of four years, aliquots were thawed and used in quintuplicates as reference samples on each 96-well plate in numerous studies conducted with the Biocrates AbsoluteIDQTM p180 kit. To ensure high reproducibility, samples were prepared with a Hamilton Microlab STARTM robot. In a targeted metabolomics approach MRMs were acquired with a Sciex API 4000 instrument using flow injection analysis and liquid chromatography. The data were used to statistically model the course of the metabolite concentrations over a time range of four years. The platform design with a continuously assayed reference sample is well suited for HTS metabolomics control studies in terms of assay consistency. Our platform showed a good performance with variation coefficients below 25 % for low concentrated analytes and better than 10 % for the highly concentrated ones. Most of the metabolites analysed with the Biocrates AbsoluteIDQTM p180 kit were remarkably stable when stored at -80 °C for four years. Particularly, acylcarnitines, lysophosphatidylcholines, phosphatidylcholines, and sphingomyelins exhibited only minor and non-significant variations below 10 %. Merely some (biogenic) amino acids and the hexoses showed significant alterations in the metabolite levels. Since highest number of significant alterations were observed within the amino acids, special attention must be paid to data of this metabolite class especially in longitudinal studies where data from samples with long storage times are compared. Correction procedures, e.g. determination of a slope, which can be used as normalisation factors in retrospective or longitudinal studies, shall be developed from the present project. This is the first report on the time-course of absolute metabolite concentrations during long-term storage of samples for metabolomics studies.

POSTER 063

Easy Blood Sampling for Metabolomics by Using Dried Blood Spots

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Sampling of blood plasma or serum for metabolomics measurements needs special procedures and medical assistance. Dried blood spots (DBS) offer some essential advantages over fluid samples. (1) Sampling is minimally invasive and easy to perform, even by probands at home. (2) Low sample volume is required. (3) Shipment and storage are easy. (4) Whole blood markers can be detected. DBS samples are already successfully applied in diagnostics. The targeted metabolomics kit AbsoluteIDQTM p180 (Biocrates) has been developed and validated for the quantification of metabolites in human plasma samples. Up to now, no study has applied this targeted metabolomics assay to dried blood spot samples.

We therefore thoroughly tested the application of the p180 kit on DBS. To assure homogenous sample properties, venous EDTA-blood from one male and one female volunteer was collected. Aliquots of freshly taken blood were spotted onto Whatman 903TM filter paper. The samples were dried at room temperature for 4 h to receive DBS. The DBS were either processed directly on the day of preparation or stored until use as described below. 3-mm diameter disks were punched out of the centre of the DBS and immediately transferred to the wells of the p180 kit filter plate for further processing. Mass spectrometric measurements were performed on a Sciex API 4000 using FIA-MS/MS (flow injection analysis tandem mass spectrometry) as well as LC-MS/MS (liquid chromatography tandem mass spectrometry). We provide a sensitive and reproducible method for targeted metabolomics measurement of DBS by p180 kit. The assay allows the simultaneous extraction and quantification of 188 metabolites of different substance classes including amino acids, biogenic amines, free carnitine, acylcarnitines, hexoses, glycerophospholipids, lysophosphatidylcholines, phosphatidylcholines, and sphingolipids. We successfully applied and partially validated the method. Factors that could potentially affect the quantification method (haematocrit, anticoagulant, ion suppression, sample handling, storage condition) were investigated. Despite a much smaller analysis volume of DBS compared to plasma, those factors had no significant influence on the ability of the method to quantify most of the metabolites in DBS, and did not restrict the usability of the method. Even the direct application of low amounts of blood to pre-cut filter paper disks was shown to be a feasible and reproducible method which can be very useful when only small sample volumes are available. Furthermore, the observed matrix effects do not influence the reproducibility or the assay linearity. Long-term storage of DBS turned out to be possible with best overall metabolite stability when samples were kept at -80 °C under dry conditions. Our results show the potential of the new DBS matrix application for further development of metabolomics technologies and diagnostics. Application of DBS enables a decentralized sample taking and uncomplicated shipment to the clinical study centers. DBS are suitable blood samples for targeted metabolomics measurement by AbsoluteIDQTM p180 kit.

POSTER 065

An evaluation of the inter-laboratory reproducibility of a targeted metabolomics platform for analysis of human serum and plasma

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A critical question facing metabolomic research is whether data obtained from different centres can be effectively compared and combined, enhancing the statistical power and return-on-investment of metabolomics studies. One important part of addressing this question is the assessment of the inter-laboratory precision (reproducibility) of the analytical protocols used. Currently many laboratories are utilising targeted metabolomics platforms, such as the AbsoluteIDQTM p180 in many human cohort studies like the EuroMotor, EPIC, HELIX. Several test materials, including the NIST reference human plasma (SRM 1950), were distributed to six laboratories and independently analysed using the AbsoluteIDQTM p180 Kit (Biocrates Life Sciences AG). The AbsoluteIDQTM p180 allows the targeted analysis of amino acids, biogenic amines, acylcarnitines, sphingolipids and glycerophospholipids. Amino acids and amines are analysed quantitatively with LC-ESI-MS/MS, while lipids and acylcarnitines are analysed semi-quantitatively, using flow-injection analysis (FIA) ESI-MS/MS. Each laboratory followed

the manufacturer protocol but used different LC-MS/MS platforms (varying combinations of instruments and HPLC/UPLC). Some of the test materials were repeated collections from the same individuals using different plasma anticoagulants or serum collection. Preliminary analysis was performed on data from 20 test materials (serum and plasma from healthy controls) obtained from the five laboratories reporting thus far. After excluding 12 metabolites (of 189) not consistently detected in all laboratories, a high degree of analytical precision was observed across for metabolites measured quantitatively. Normalisation of measurements to the profile of a standard reference material obtained in each laboratory run significantly improved the inter-laboratory precision of metabolites measured via FIA. After normalisation, the majority (typically ~75%) of metabolites in each test material exhibited an inter-laboratory coefficient of variance (CV) of <10%. Approximately 90% of metabolites exhibited an inter-laboratory coefficient of variance (CV) of <20%. Ongoing analysis will also assess the impact of highly lipidic samples and varying anti-coagulant on precision, as well as the accuracy of measurement of specific quantified metabolites. This is the first inter-laboratory assessment of this metabolomics platform, providing critical information for users to interpret these data appropriately.

POSTER 066

From Mouse to Man – Metabolomics in Translational Research

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Metabolic phenotyping comprises the quantification of endogenous metabolites in biofluids, cells, and tissues. It provides insights into pathways and biological processes, which is important for the understanding of disease phenotypes. It also allows the identification of biological surrogate markers, which can serve as early disease indicators and therapeutic markers for the evaluation of treatment effects. As metabolic markers are not species-restricted, the concept of metabolic phenotyping is highly applicable for translational research. Species independence allows the use of established animal and cell culture models for various diseases within a preclinical context. However, differences in the metabolic set-up of study organisms compared to humans needs to be taken into consideration to prevent misleading conclusions from otherwise valid experimental designs. According to widely differing requirements in the field of metabolomic research, mass spectrometry based targeted metabolomics platform for multi parameter analyses was developed which comprise varying sets of metabolites. The need of minimal sample volumes and the flexibility to varying biological matrices as well as analyte quantification over a wide spread range of concentrations is a high advantage of these ESI-MS/MS methods. By the use of this platform, over 600 analytes can be quantified easily and quality controlled with high reproducibility and accuracy. This set of analytes consists of the following classes: acylcarnitines, amino acids, biogenic amines, eicosanoids, (lyso-) phosphatidylcholine, (lyso-)phosphatidylglycerols, (lyso-)phosphatidylethanolamines, phosphatidyl-serines, sphingomyelins, ceramides, steroids, neurotransmitters, bile acids, energy metabolism, oxysterols and vitamins. Overall, the validity of metabolic phenotyping will be demonstrated, despite or even because of species-dependent characteristics. It has the potential to explain why findings in animal models cannot always be directly translated into clinical settings and might, therefore, facilitate the establishment of suitable models of disease. Here, this is demonstrated on basis of the analysis of bile acids in mice and men. Several species individual and comprehensive bile acids were found in humans and mice. Nevertheless, there are major differences in the bile acid composition in both species. Besides the mouse-specific bile acids (Muricholic acids), we have found differences in conjugation patterns of bile acids. In humans the analysis of the bile acids showed more Glycine-conjugated bile acids whereas in mice Taurine-conjugation was dominant. This finding could be related to species-dependent detoxification pathways. In contrast both species show catalytic activity of CYP3A of the cytochrome P450 superfamily, which is important for studies of the drug metabolism. Another very interesting aspect which was found in our bile acid study was that we could easily separate female and male mice by their bile acid profile. All these aspects and findings underline the importance of thoroughly choice of a suitable research model dependent on the question of interest and for translational research. Bile acids analysis could improve selection of correct models for translational research and reduce numbers of animal tests.

POSTER 067

Metabolomics based exhaustive profiling of Japanese Soy Sauce revealed the significant involvement of dipeptides on slight taste differences.

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Soy sauce is one of the most important fermented seasoning in Japan. It is well known that the specific flavor of Japanese soy sauce would be basically formed by a combination of glutamic acid, salt, and sugar through the heating treatment after fermentation. However they would not be enough to explain very much naïve and slight differences of flavors between several kinds of fermented Japanese soy sauces. The additional unknown components must be involved for slight difference among various Japanese soy sauces. Previously some dipeptides which might effect on taste several fermented foods were reported. The objective in this study is to investigate the correlations between the dipeptides and the taste differences among soy sauces. To analyze the dipeptides, an analytical method using liquid chromatography/tandem mass spectrometry (LC/MS/MS) in multiple reactions monitoring mode was constructed. Additionally, hydrophilic small metabolites were analyzed by means of a conventional method by GC/Q-MS after derivatization. Sample soy sauces were subjected to both GC-Q-MS and LC-QQQ-MS to yield metabolome information of hydrophilic small metabolites and dipeptides respectively. The obtained mass spectrometry data were merged to organize data matrix. Sample soy sauces were subjected to quantitative descriptive analysis by skilled veterans to obtained quantitative sensory value. Both metabolome data matrix and QDA sensory data matrix were subjected orthogonal partial least square projection to latent sturcture regression (OPLS regression) to investigate relationship between dipeptides profile and QDA sensory value. Based on an analytical method using liquid chromatography/tandem mass spectrometry (LC/MS/MS) in multiple reactions monitoring mode, we detected 237 dipeptides, the largest number ever detected in soy sauce research. Next, orthogonal projections to latent structures regressions were performed. The data matrix of components, including dipeptides and other low-molecular-weight hydrophilic components obtained from gas chromatography/mass spectrometry (GC/MS), served as explanatory variables (366 in total), whereas a sensory data matrix obtained using quantitative descriptive analysis served as the response variable. The accuracy of models for the sweetness and saltiness differences constructed using the LC/MS/MS and GC/MS data matrix were higher than did models constructed using only the GC/MS data matrix. As a result of investigation of the correlation between the dipeptides and taste differences among soy sauces by using variable importance in the projection (VIP) score, many dipeptides showed the high correlation with taste differences. Specifically, Ile-Gln, Pro-Lys, Ile-Glu, Thr-Phe, and Leu-Gln showed the high VIP score on sweet differences. This study is the first report that reveals the correlations between the dipeptides and taste differences among soy sauces. In Japanese soy sauces, 237 dipeptides were detected. Among them several peptides were correlated to difference of flavor.

POSTER 069

Cobalt-60 radiation alters the metabolome of NIH7001 rodent chow diet with implications for changes in model responses

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To obviate problems associated with microbial contamination of rodent diets, diet manufacturers have introduced irradiation of diets with cobalt-60 radiation. However, in many cases these irradiated diets have changed the outcome of rodent model experiments. The fecal microbiomes of rats treated with irradiated diets were significantly different from those from animals receiving diets that had not been irradiated. The goal of this study was to examine the effects of the cobalt-60 radiation on radiation-sensitive components of the diet. The diet was prepared as a single batch by Harland-Teklad and divided into two - one half was exposed to Co-60 radiation and the other not. Samples of NIH7001 diet were taken and frozen during the course of a study. Aliquots of diet (1 g) were extracted with 5 ml ice-cold 80% methanol. Extracts were evaporated to dryness under vacuum, reconstituted in 1 ml of double distilled water and centrifuged to remove any particles. Aliquots (1 ul) were injected using an Eksigent 415 NanoLC onto a 0.5 cm x 75 mm ID C18 pre-column cartridge; bound metabolites were eluted with a 1 ul/min 0-80% acetonitrile gradient in 0.1% formic acid onto a 15 cm x 200 um ID ChipLC column at 45oC. Eluates were passed through a nanoESI source into an AB Sciex 5600 TripleTOF mass spectrometer.and positive and negative ion MS and MSMS spectra recorded. XCMS analysis of the nanoLC-MS data revealed extensive differences between the non-irradiated and irradiated diets for both negative and positive ions. A complete separation was observed using 2D-Principal Components Analysis (PCA). This held true when XCMS data were exported to MetaboAnalyst and were normalized by total ion current and centered using interquartile range and Pareto scaling. Additionally, 2D-Partial Least Squares Discriminant analysis (PLSDA), and Random Forest analysis allowed further aspects of the separation. Detected ions were also subjected to Mummichog analysis, a new feature available with the online version of XCMS. This program bypasses the export of ions to be matched to records in metabolomics databases and then mapping them to pathway sites. Mummichog analysis assesses the pathways/groups predicted by the ions detected in an analysis. For the positive ions, the identified pathways were urea and amino group metabolism (P=0.00233), drug metabolism (P=0.00257), ubiquinone biosynthesis (P=0.00317), leukotriene metabolism (P=0.00553), vitamin E metabolism (P=0.01736), sialic acid metabolism (P=0.02145), vitamin B6 metabolism (P=0.04141), bile acid biosynthesis (P=0.04715) and histidine metabolism (P=0.04949). The most significant chemical groups were substrates of CoA (P=0.000006), glutathione metabolites (P=0.00014), bile acid metabolites (P=0.00016), histidine and S-adenosyl methionine metabolites (P=0.01251) and non-gonadal steroids (P=0.01623). For the negative ions, the identified pathways were linoleate metabolism (P=0.00114), valine, leucine and isoleucine degradation (P=0.00355), drug metabolism - other enzymes (P=0.00817), TCA cycle (P=0.00817), prostaglandin formation from dihomo gamma-linoleic acid (P=0.01021), fatty acid oxidation in peroxisomes (P=0.01021), ubiquinone biosynthesis (P=0.01328), tryptophan metabolism (P=0.01380) and butanoate metabolism (P=0.01797). The most significant chemical groups were aromatic metabolites (P=0.00091); S-adenosyl methionine metabolites (P=0.00131), tryptophan metabolites (P=0.00927) and non-gonadal steroids (P=0.01553). Mummichog analysis also served to collect all the 13C isotope and adduct ions (Na+, K+, Cl-, formate and acetonitrile) observed for each metabolite. These data also reveal that this diet contains many physiologically relevant metabolites. Radiation of a common rodent diet with cobalt-60 causes the destruction and/or modification of nutritionally and physiologically important metabolites.

POSTER 071

Metabolite changes during makgeolli fermentation manufactured with nuruk, koji and decoenzyme

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Makgeolli, is a Korean traditional alcoholic beverage. The taste and flavor of makgeolli are mainly determined by the metabolic products such as free sugars, amino acids, organic acids, and aromatic compounds which are produced during fermentation process of raw materials by the microorganisms present in nuruk, a Korean fermentation starter. In this study, we brewed makgeolli using nuruk (pressed type formed various fungi in a nature state), koji (scattered type inoculated single strain) and decoenzyme (glucoamylase produced by Rhizopus). During the fermentation, temporal changes in the metabolites were investigated by metabolomics approaches. The aim of this study was to investigate metabolic

changes during makgeolli fermentation to determine whether the microbial composition of nuruk affect to the quality of makgeolli. To extract metabolites for UHPLC-Q-TOF MS analysis, 0.9mL of 50% MeOH (internal standard reserpine, 10 ppm) was added to 0.1 mL of makgeolli; after vortexing for 5 min, the mixture was kept at 4°C for 16 hr. Next, centrifugation was performed at 14,000 rpm at 4°C for 20 min; the supernatant was then collected and the metabolites were extracted. The metabolites of makgeolli were simultaneously analyzed by liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS). The metabolites data were processed by partial least squares-discriminant analysis (PLS-DA) as multivariate analysis. Separating main variables of each makgeolli on PLS-DA loading plot were tentatively identified through molecular weight, retention time, and MS/MS fragmentation pattern analyses. The makgeolli samples taken at different fermentation starter (nuruk, koji and decoenzyme) were clearly distinguishable in the score plot generated by combining PC1 (47.30% of the total variance) with PC2 (10.90% of the total variance). Makgeolli fermentation processes are carried out using various kinds of microorganisms, including fungi, yeast, and bacteria. Theses microorganisms produce amino acid, organic acid, as well as various metabolic compounds that control the taste and flavor of makgeolli. Major microorganisms in nuruk were Saccharomyces cerevisiae, Aspergillus oryzae, Penicillium sp., Wickerhamomyces anomalus and Rhizopus oryzae. Microorganism in koji was present only Aspergillus oryzae. Makgeolli metabolites profiling were affected by microbial composition of fermentation starter. As a result, changes in these metabolites allowed us to distinguish among nuruk makgeolli samples with different fermentation periods (1, 3, 5, 8, and 9 days) on a PLS-DA score plot. Makgeolli using the nuruk in which the kind of fungi is various showed the aspect, considerably changed according to the fermentation period in the partial least square discriminant analysis. The significantly different metabolites between three groups might to be small peptides, purine base and vital nutrient organic compounds. This study revealed that mass based metabolites profiling was useful in helping to understand the metabolite differences by microbial composition of fermentation starter. Metabolites based on LC-ESI-Q-TOF-Mass spectrometry technique may be of interest tool for study in estimation of makgeolli quality.

POSTER 072

Profiling of quality parameters and secondary metabolites to explore the metabolome of apples and juices from more than 60 cultivars

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Apple (Malus domestica) constitutes one of the most frequently consumed fruits worldwide and thus a significant part of the human diet. Metabolites such as polyphenols influence the nutritional value and beneficial effects of fruit. Their amount in apple as well as quality attributes like colour, size, firmness and flavour vary, among other factors, according to variety. In this study, a comprehensive characterization of both primary and secondary metabolites and established quality attributes was performed on apples and cloudy monovarietal juices from 64 different commercial, old and red-fleshed cultivars. The characterization of cultivars in terms of health and sensory properties not only for direct consumption but also as raw material for juice production will constitute the output of the performed work. All 64 cultivars included in the study were grown in the same experimental field under identical conditions during the 2013 season at Laimburg Research Centre (altitude 220m). After 60±10 days of storage (2°C and 90% humidity) the weight, soluble solid content, pulp firmness and titrable acidity were registered and freshly pressed cloudy monovarietal juices were prepared. Primary quality parameters (turbidity, soluble solid content, titrable acidity and colour) were evaluated on juices. For both pulps and juices pectin content was gravimetrically quantified and individual sugars and acids were analyzed with ion chromatography. For peels, pulps and juices total antioxidant activity, total phenolic content and total anthocyanins were measured and individual polyphenols analyzed by UHPLC-ESI-QqQ-MS in MRM mode. Large ranges of

variation for all measured parameters characterized the dataset. The weight of the apples ranged from 100 to 350 g, while their firmness varied from 3 to 8 kg/cm2. The juices showed turbidity ranging from 58 to 2827 NTU. The content of pectin ranged from 2 to 12 g/100g in the pulps and up to 3 g/100mL in the juices. Total soluble solid content ranged from 9 to 16 °Brix, but the most varying parameter was total acidity which spanned from 2 to 14 g/L of malic acid. PCA of the above parameters and primary metabolites on both pulps and juices showed clear grouping of red-fleshed varieties characterized in particular by their higher acidity, but also lower turbidity, higher firmness, juice yield and sugar content. Commercial and old varieties instead showed a broader distribution of these attributes. However, old varieties reached higher acidity values compared to commercial ones. Old and red-fleshed varieties showed higher total phenolic content and total antioxidant capacity in both pulps and juices while for peels higher ranges of variation were present. PCA of individual polyphenols showed for both peel and pulp a discrimination of red-fleshed and old varieties due to their relative amounts of anthocyanins, flavan-3-ol and procyanidins. The peel of commercial varieties mainly fell between these extremes, with some varieties containing higher amounts of anthocyanins and flavonols. In the case of pulps, the majority of commercial varieties were clustered together for their generally lower amount of polyphenols and lower biological variation. In PCA of the juices, the differentiation was less clear due to the general and indiscriminate decrease of polyphenols. However, some red-fleshed varieties that produced a bright red juice could be recognized due to higher amounts of anthocyanins as expected. In addition, some varieties were differentiated by higher level of polyphenols. Comprehensive characterization of quality attributes, primary and secondary metabolites of apples and juices from old, commercial and red-fleshed apple cultivars.

POSTER 073

Effect of various cooking methods on content, oxygen radical absorbance capacity, and bioaccessibility of caffeoylquinic acids in Ligularia fischeri Turcz

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Ligularia fischeri Turcz known as 'Gom-chi' in Korea, a member of the compositae, is stably supplied and mostly consumed in Korea. Gom-chi has been recognized as plant predominantly contains caffeoylquinic acids. Caffeoylquinic acids (CQAs), as a generally known group of antioxidant compounds, are known to provide diverse biological functions such as a scavenging of free radicals, and resisting pathogenic insects. Owing to various functions of CQAs, Gom-chi is known to contain a significant amount of CQAs and has been used as traditional medicine. Thus, the objective of the current study was to determine the CQA content in Com-chi according to various cooking methods to estimate bioaccessibility of CQAs, and to measure the antioxidant capacity Mono-, di-, and tri-CQAs were identified and quantified by using an ultra-performance liquid chromatograph-photodiode array detector. In vitro biomimick system was performed for assessing the bioaccessibility of CQAs. The free radical scavenging capacity was measured by ORAC assay, expressed as trolox equivalence (TE). The amount of 5-CQA, 4-CQA, 3-CQA, and 3,4-di-CQA were 136.72, 2144.44, 16.81, and 421.93 μg, respectively, in g of fresh Gom-chi. Both the content of CQAs and TE value were generally reduced by various cooking methods. The highest amount of CQAs and TE was observed in microwaving for 3 min and pan frying for 3 min, respectively. The ranking of total CQAs were found in the order of microwaving 3 min (239.96 μg/g) > microwaving for 2 min (206.11 μg/g) > blanching for 3 min (191.94 μg/g) > blanching for 5 min (180.32 μg/g) > pan for frying 5 min (161.20 μg/g) > pan frying for 3 min (115.83 μg/g). The bioaccessibility of total CQAs were 16.42, 17.64, 14.39, 13.29, and 12.43 (%) in fresh, blanching for 3 min, blanching for 5 min, pan frying for 3 min, and pan frying for 5 min, respectively. Among the cooking methods, blanching for 3 min had the highest amount of CQAs after digestion. The total CQAs in microwaved Gom-chi were not detected, indicating that CQAs showed low bioaccessibility. In conclusion, cooking has an significant role in the content, oxygen radical absorbance capacity, and bioaccessibility of CQAs from Gom-chi. Fresh Gom-chi contained the highest amount of each CQA, and the scavenging ability of peroxy free radicals. Among various cooking methods, microwaved Gom-chi had the most CQAs, but barely digestible in simulating the human GI tract. Among cooking methods, Gom-chi with blanching for 3 min provided the greatest bioaccessibility of total CQA. Therefore, blanching for 3 min may be considered a useful preparation method for enhancing absorption and biological functions of CQAs from Gom-chi. To increase absorption

and antioxidant capacity of bioactive component such as CQAs from Gom-chi, this study suggest optimal cooking method.

POSTER 074

Metabolite profiling of soybean fermented with Lonicera caerulea and Cudrania tricuspidata and its anti-obesity effects in vivo.

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Fermented soybean products have more health benefits, such as antioxidant, anti-obesity and pancreatic lipase inhibitory activities, than non-fermented soybean in vitro and in vivo. Combination of several natural products can exhibit synergistic biological effects on anti-obesity and anti-diabetes via various mechanisms. However, there is lack of researches on fermented soybean with added natural substance. In this study, we tried to investigate metabolite changes and bioactivities of soybean fermented with Lonicera caerulea and Cudrania tricuspidata by Bacillus subtilis and to examine the anti-obesity effects of fermented soybean mixture (FSM) by metabolite profiling of plasma in high-fat diet (HFD)-induced obese mice. Soybean (30g) was fermented with L. caerulea (1%, w/w) and C. tricuspidata (5%, w/w) by B. subtilis for 60h (FSM60). Metabolite profiling of non-fermented soybean mixture (FSM0) and FSM60 was performed using ultra-performance liquid chromatography–quadrupole-time–of-flight mass spectrometry (UPLC-Q-TOF-MS) and total phenolic content (TPC), total flavonoid content (TFC), ABTS, and pancreatic lipase activity were also measured. In animal study, mice were divided into 4 groups; normal-diet group (ND), HFD group (HD), HFD with FSM0 administrated group (HFSM0), and HFD with FSM60 administrated group (HFSM60). After experimental periods, we observed clinical and histological changes induced by HFD and FSM administration in C57bl/6 mice model. The plasma obtained from mice was analyzed using UPLC-Q-TOF-MS combined with multivariate analysis. Soybean fermented by B. subtilis (FS) and FSM were clearly separated on partial least-squares discriminant analysis (PLS-DA) according to fermentation (0 and 60h) and adding substances. We selected and identified significantly altered metabolites by fermentation. Soybean isoflavone glycosides were converted into their aglycones due to fermentation which were observed both in FS and FSM. In FSM0, high levels of flavonoid glycosides such as anthocyanins, flavones, flavonols, prenylflavonoids, and isoflavones derived from L. caerulea and C. tricuspidata were observed. These glycosides were also converted into their aglycones during fermentation process. As a results, FSM60 contained higher levels of aglycones, such as luteolin, quercetin, daidzein, glycitein, and genistein than FS60. TPC, TFC, antioxidant, and pancreatic lipase inhibition activity were higher in FSM than FS under the influence by adding L. caerulea and C. tricuspidata with a variety of active constituents. In obese mouse, the levels of cholesterol and triglycerides, weights of body, subcutaneous fat, adipose tissue, spleen, liver, and kidney, as well as adipocyte area altered by HFD, were improved by FSM0 and FSM60 consumption. Especially, body weight change and adipocyte area were more significantly decreased in HFSM60 than in HFSM0 group. From UPLC-Q-TOF-MS data sets, 26 metabolites including lysophosphatidylcholine, lysophosphatidylethanolamine, and 4 non-identified metabolites were selected as the different metabolites that affected by the clinical change. Most of lysophospholipids were remarkably influenced by both FSM0 and FSM60 administration as indicated that these metabolites could be potential biomarker candidates to inhibit HFD-induced obesity. Soybean fermented with various natural substances could possess a synergistic biological effect on anti-obesity by metabolite alteration.

POSTER 075

MS based Metabolomic Approach to Investigate Effective Food Components against Hepatic Fibrosis

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The fermented soybean products have been used traditionally as a great source of nutrition, providing protein and essential amino acids that are insufficient in the grain-based Korean diet. Moreover, these fermented foods exhibit diverse physiological characteristics such as obesity-preventing effects, anticancer, anti-diabetic activities, enhanced immune function and hepatoprotective effects. In the present study, we investigated the metabolite profiles of fermented soybean products according to a-SMA-expression suppressing effects by metabolomics, for the purpose of discovering effective bioactive substances.This study highlights that comprehensive metabolite analysis of traditional soybean products provides an improvement the liver function and useful information for better understanding the factors related to fermented soybean products metabolites effects against hepatic fibrosis. Twenty-four certified traditional Doenjang samples used in this study were obtained across all the areas in Korea. For metabolites analysis, UPLC system (Agilent 1290 Infinity) coupled to a Q-TOF mass spectrometer (Agilent 6520 with Jet Stream Technology, Agilent) was used to analyze metabolites of soybean products. All MS data were extracted using the MPP software package (Agilent). Isoflavone contents of Doenjang were analyzed using Agilent technologies HPLC 1200 Series (Agilent Technologies) equipped with reverse phase C18 column. MS data were also aligned and normalized by the MassHunter Mass Profiler Professional software (v. B. 02. 01). The resulting data sets were then imported to SIMCA-P version 12.0 (Umetrics, Umeå, Sweden), and PCA and PLS-DA were used to discriminate among each group. In this study, we investigated the metabolites profiles of Doenjang according to a-SMA expression suppressing effect through mass spectrometry based metabolomics. Also the relationship between Doenjang metatbolites and a-SMA expression suppression effect was identified. PLS-DA score plots showed that the doenjang samples were clearly separated into two parts: (i) dependences on natural fermentation and (ii) single strain inoculation before the fermentation of doenjang with good statistical indication values. The Doenjang samples with higher a-SMA expression inhibiting activity were characterized by higher levels of proline, 4-methylen-L-glutamine, valine, cycloleucine, Poster aminobenzoic acid, and soyasaponin III compared to those with lower inhibition activities. Also, the levels of most of the amino acids and isoflavone aglycone are much higher in the traditional Doenjang, especially higher a-SMA inhibitory activity, whereas higher levels of isoflavone glycosides, glycitin, daidzin, and genistin were found in the doenjang samples with a short-term fermentation using a single strain. The hydrolysis of soy protein and the deglycosylation of isoflavone glycosides convert them to amino acids and aglycones through microbial activities in the fermentation process. Therefore, higher levels of isoflavone aglycones and BCAAs were pointed by bioactive substances for the hepatic fibrosis in this study. In the efforts to find out traditional Doenjang metabolites related to liver functions, consequently branched-chain amino acids, Poster aminobenzoix acid, 7,4-dihydroxyflavone, soyasaponin and isoflavone aglycone were found to be higher in Doenjang group showed higher a-SMA expression suppression activity. The present study highlights that comprehensive metabolite analysis of traditional Doenjang provides an improvement the liver function and useful information for better understanding the factors related to Doenjang metabolites effects against hepatic fibrosis. These results will provide guidelines for the improvement in liver function instilled by traditional soybean products.

POSTER 076

Fruit industry by-products: what are we throwing away?

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Fruit processing produce large amounts of by-products in the world and a great percentage of it is managed as waste evoking economical and environment concerns. Additionally, most of these by-products are not well characterised and are generically considered a high sugar low nitrogen substrate.

On the other hand, in a contemporary perspective these by-products could be employed for developing new added value products. In order to enable this approach, we characterised nine industrial fruit and vegetable by-products using metabolomics tools and assessed their nutritional parameters in order to determine their potential application as a possible raw material for bioconversion and/or biorefinery. The fruit industry by-products assessed were mainly fruit and vegetable residues from juice production, including apple pomace, orange pomace, three different kiwi-fruit by-products, grape pomace, olive cake and carrot pomace. A fast alkylation reaction based on methyl chloroformate (MCF) and the traditional trimethyl silyl (TMS) derivatizations were used to characterize the composition of amino and non-amino organic acids, and soluble sugar and derivatives, respectively. After derivatizations, the samples were analysed by GC-MS. Additionally, the content of acid and neutral detergent fibers; ash; nitrogen; moisture; crude protein; total fat; soluble sugar; pH; phosphorus; magnesium; organic matter; digestibility of organic matter (DOMD); metabolisable energy; non-structural carbohydrate and starch were also determined for each substrate. The nutritional parameters between substrates varied widely. Acid detergent fibre varied between 18.370.8%; neutral detergent fibre 14.869.1%; nitrogen 0.62.2%; moisture 4489%; crude protein 3.612.9%, crude fat 3.612.9% and soluble sugar 0.150.2 g/L. These data show that some substrates present indeed a high sugar low nitrogen composition, although this characteristic does not fit all of them and clearly do not represent all the nutritional constituents. For example, olive cake is a low sugar high fat and high fiber substrate. GCMS analyses were able to detect over 400 compounds and metabolite identifications varied from 77 to 103 using our in house MS libraries of standards. Apple, orange and carrot pomaces were characterised by a diverse range of amino acids (0.02 to 0.97 µmol/ml), including essential amino acids such as leucine, valine, and tryptophan. Additionally, a considerable number of phenolic acids were observed in the majority of the byproducts (eg. sinapic acid; syringic acid and trans-cinnamic acid). Due to the widespread use of this class of compounds in pharmaceutical and chemical fields, we are now expanding our MS library to increase the number of phenolic acid identifications. Moreover, all analyzed by-products showed a considerable number of unknown compounds, which represent a potential source of novel metabolites, not previous described elsewhere. First comprehensive metabolite profiling of fruit and vegetable by-products to determine their potential for bioconversion and/or biorefinery processes.

POSTER 078

What are we eating? Comprehensive analysis of food metabolites and natural products using eight metabolomics platforms

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Foods consumed by humans can be assessed by the same techniques as endogenous metabolites. This study uses MS-based untargeted metabolomics for the analysis of both food metabolites and natural products found in certain food plates, focusing on the identification and annotation of small molecules found in foods. For the first time, we here use eight metabolomics platforms (four different LC-MS and four GC-MS methods) to provide a comprehensive analysis and characterization of metabolites and natural products in three different food plates. These food plates were selected on large differences in dietary components, with a fast food meal (USA plate), a California plate (based on USDA MyPlate dietary recommendations) and the Davis plate, inspired by Korean cuisine. All food items on each plate were photographed, homogenized and then lyophilized (except for volatile profiling); the “CA plate” and

the “USA plate” were also carefully balanced with respect to dietary parameters. The LC-MS platforms were: 1) Lipidomics 2) HILIC 3) Reverse phase all on an Agilent 6530 Quadrupole Time-of-Flight (QTOF). Flavonoid analysis was also done using multiage-stage mass spectrometry (MSn) on a linear ion trap (LTQ). The GC-MS platforms were trimethylsilylated using: 1) GC-quadrupole MS 2) GC-TOF MS 3) GC-QTOF 4) volatile analysis by GC-TOF MS (without derivatization). Diverse data processing methods were used including ChromaTOF deconvolution with BinBase database use, MS-DIAL data processing with MassBank and LipidBlast database queries, and accurate mass analysis in GC-QTOF and LC-QTOF MS. Three food plates from different geographical regions were selected in this study to investigate the food metabolites and natural products. A non-targeted metabolomics approach was used to annotate and identify several metabolites across eight platforms. Primary and polar food metabolites were profiled using the GC-TOF-MS. Over 500 annotations were made, of which about 200 were identified as known metabolites. These known compounds include amino acids, dipeptides, mono- and di-saccharides, sugar alcohols, sterols, organic acids, nucleosides, and free fatty acids. Volatile organic compounds were analyzed using GC-TOF-MS coupled with a thermal desorption unit. Over a hundred metabolites were annotated by BinBase. Such metabolites are related to short chain fatty acids and aroma volatile compounds. GC-QTOF-MS data was used to annotate unknown food metabolites and natural products. Using the lipidomics method, over 200 lipids were annotated using LipidBlast. The triglycerides levels across all three plates were the highest. Using the HILIC and reverse phase methods, over 100 metabolites were annotated by MS/MS mass spectral library search in NIST 14, ReSpect, Mass Bank, and METLIN. Another 100 polyphenol and flavonoids were annotated from the platform using MSn analysis on the LTQ. Data was annotated using an in-house reference flavonoid and natural product ion tree library. Using Mass Frontier 7.0, experimental ion trees from the most abundant ions from each food plate samples were queried against the user ion tree library. Tree match similarity score of 700 and above were used to compile a list of potential compounds used for annotation of flavonoids in each food plate. Overall, food plate samples display a wide range of food metabolites and natural products that play a major role in the entire food composition. The analysis of such samples exemplifies that nutritional food metabolites and natural products are much more abundant in the California and Davis plates. An insight into the diversity of three different food plates using comprehensive Foodomics analysis.

POSTER 079

Which wine will I like best? High resolution polyphenol profiling of red wines using microLC-MS/MS

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Red wine has been of great interest to chemists and biologists due to its high abundance of polyphenols, some of which were reported to have potential benefits for human health. The flavonoid composition also plays a great role in the color stability and taste of the red wines. Therefore, understanding the composition of red wines is very important. However, most wine studies only measured a few abundant compounds in red wines; comprehensive profiling of polyphenols contents is still needed. Here we optimized a high-throughput microLC-MS/MS method for untargeted profiling of polyphenols in red wines. An Ekigent micro LC 200 was coupled to an AB SCIEX TripleTOF 5600 for the data acquisition. A standard mixture of 15 phenol compounds was used to develop the LC/MS method. Mobile phase A and B were water and acetonitrile, respectively, with 0.1% acetic acid. Three C18 columns of 1 mm I.D. and 5 cm length were examined, with 11 gradients of different flow rates and gradient lengths. The optimum column and method was established by evaluating the peak intensity, peak capacity and peak number. Six red wines were tasted by a panel of average people and then extracted with ethyl acetated and measured using the optimized method. Data was processed by MS-DIAL, NIST MS PepSearch and Devium. Among the three columns we have tested, the Kinetex 2.6 μm proved to perform best in terms of peak intensity and peak capacity for the standard mixtures. A four min gradient from 10% to 90% B at flow rate of 0.05 mL/min provided the highest number of peaks for a preliminary test wine sample and

therefore was applied to other extracted wine samples. For comparison we also acquired SWATH-type MS/MS data in a twelve min gradient. From the MS/MS spectra in positive and negative mode, 264 compounds were annotated with a Rev-Dot score higher than 800 when comparing spectra to NIST 14, Metlin, MassBank and ReSpect database. To further validate the annotation results, we confirmed 18 annotations by injecting the corresponding authentic standards. We also annotated novel isomeric compounds. For example, in addition to the well-known cis- and trans-forms of resveratrol, two further isomers of this flavonoid were observed. Using peak heights as semi-quantitative measure of flavonoid abundances, large differences in the metabolite profiles was found between the six red wines using unsupervised multivariate statistics and univariate analyses. Specifically, trans-resveratrol was 3- to 6-fold more abundant in Pinot Noir and Merlot than in the other four wines. An OPLS model was built between the tasting scores and metabolite peak intensities. If trained sensory panel and a large variety of wine samples are available, important features affecting the taste of wine can be found. Therefore, the method is also applicable to sensory studies and quality control of red wine production. Compared to most of published LC/MS methods in red wine studies, our method is faster, with a cycle time of 5.5 min; greener, with mobile phase consumption of 0.275 mL per sample; and more comprehensive, with more than 200 compounds annotated in a single run. Wine-omics is near! The development of a faster, greener and more comprehensive microLC-MS/MS method for polyphenol profiling of red wines

POSTER 080

Metabolomic characterization of Merlot wine composition: Objective measurements of sensory properties

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Maturity is known to have significant impact on the composition of wine grapes, particularly during the exponential accumulation of soluble solids. Ethanol has numerous effects on wine chemistry of sensory importance including impacting the extraction of many non-volatile compounds during maceration and the partitioning of volatile compounds between the vapor and liquid phase in finished wine. Given the direct relationship between the resulting ethanol concentration and the initial soluble solids of a must we explored the relationships between grape maturity, ethanol, and the resulting sensory and chemical properties of wine. Merlot grapes from Washington State were harvested at three different maturities: ~20 Brix, ~24 Brix and ~28 Brix. Sugar addition (chapitalization) or watering-back were used to target different ethanol concentrations at each harvest while maintaining the original juice to skin ratios. Portions of each harvest were chaptalized or watered-back to obtain nine treatments: three harvest dates at three soluble solids levels, which were fermented in triplicate. A trained descriptive panel (N=14) rated the intensity of 16 aroma, 15 in-mouth flavor, 3 taste, and 3 mouth-feel attributes using a line scale. The wines and the aroma references used for training the panel were analyzed by untargeted SPME-GCMS to obtain their volatile profiles. The volatile aromatic profiles of the 27 Merlot wines were compared to the descriptive sensory analysis results. The trained panel differentiated the wines using attributes characteristic of green notes, including ‘herbaceous’ and ‘vegetal’ as well as attributes characterizing fruit notes, including ‘dark fruit’, ‘red fruit’, and ‘dried fruit’. Multivariate statistical analyses differentiated the wine treatments based on their volatile profiles and in combination with descriptive sensory data led to the identification of groups of compounds correlated with specific aroma attributes. In addition to the wines, aroma references, formulated to match specific aromas identified in the wines by panelists, were analyzed and their volatile profiles compared to the profiles of the wines. Compounds found in both the volatile profile of a reference and wines reported to be high in intensity for the aroma characterized by the reference may be strong contributors to the aroma and warrant further investigation and characterization. Ultimately, these aroma profiles help to determine the role that grape maturity and ethanol play in determining the sensory characteristics of red wines. This study is potentially the first to profile the volatile composition of aroma references to use in data mining.

POSTER 081

Characterising the effects of storage temperature on wine using untargeted metabolomics

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Wine that is exposed to elevated temperatures, particulary during storage or transport, may experience changes in its chemical composition and sensory attributes. The extent and nature of the changes vary with the wine variety and style, packaging materials, maximum temperatures, temperature profiles, humidity, exposure to light and the presence and absence of oxygen (De La Presa-Owens et al 1997, Arapitsas et al 2014). While some chemical reactions at elevated temperatures may not have an impact on wine sensory and quality at all, or might even lead to increases in some positive aroma attributes, other reactions will result in pre-mature ageing and undesirable sensory changes. Untargeted metabolomics is a comprehensive comparative tool for the study of global chemical composition and thus was utilised to characterise complex changes in heat treated wine compared to wine that was cellared under optimal conditions. To establish the impact of storage temperature on wine, two red and two white table wines were subjected to temperatures of 15oC, 28oC and 50oC for a 2 week period. Following heat treatment, all samples were stored at 15 oC and were analysed at t0, at 2 weeks from t0 and at 3 months from t0. The temperature effects on the wines were investigated using untargeted metabolomics experiments for monitoring volatile and non-volatile wine compounds by GC-MS and HPLC-MS/MS respectively; yeast metabolites including esters, alcohols and acetates by GCMS; and grape metabolites, ie stilbenes, cinnamic acids, anthocyanins, flavanols, flavonols and other phenolics, were analysed by HPLC-MS/MS. After data pre-processing, features derived from MS-analysis were combined with data obtained by (UV/Vis, MIR) spectroscopy, routine spectral measures of wine phenolics and colour, free and total SO2 as oxidation markers. Multivariate data analysis revealed the specific temperature impacts on various wine varietals and styles. Using informatic processing techniques, features of interest could de identified; these allowed us to narrow down the components in the wine that may explain the variation observed due to elevated storage temperatures. In summary, this study demonstrates the use of untargeted metabolomics analysis, in combination with spectral and sensory data, to identify key compositional changes in wine and establishes a model system of environmental heat stress based wine damage. This may be used to assess temperature thresholds and the impact of temperature on different wine styles and/or regional attributes. Wine composition, heat impact and crossing boundaries between microbial and plant metabolomics and food processing induced changes

POSTER 082

The diversity of aromatic rice varieties in the metabolomic, genetic, and sensory perspective

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Southeast Asia is the top exporter of fragrant rice, hence is a valuable source of aromatic rice germplasm. The key to breeding fragrant rice is to understand the totality of volatile compounds that describe pleasant and unpleasant aromas. Until now, only 2-acetyl-1-[yrroline (2AP) has been associated with rice fragrance. Jasmine rice is usually described as having a floral fragrance, but 2AP smells of baking bread. This suggests that other fragrant compounds must combine with 2AP to produce the floral aromas characteristic of the SEA varieties. The objective of this study is to compare the SEA premium rices with Australian breeding lines grown across different environments to be able to completely understand rice flavour in the genetic, metabolomic and sensory perspective. Nine aromatic rice varieties from SEA and Australia, and two non-aromatic varieties to serve as controls for both lack of aroma and germplasm class

were grown in three locations in Australia within tropical and temperate conditions. However, of the nine varieties, only five could be replicated in temperate conditions as the Asian varieties are more susceptible to cold conditions and are photoperiod sensitive. The samples were genotyped by GBS (Genotyping-by-sequencing) to be able to calculate genetic similarity. Metabolite profiling of volatile compounds was done using GCxGC-TOF-MS, and descriptive analysis of flavour was done with 10 trained panel members. PCA of the metabolomic profile of the volatile compounds of rice showed distinct clustering according to germplasm classification along PC1, while aromatic vs nonaromatic samples separated along PC2. There was no apparent clustering of the samples based on the planting location, indicating that though differences in environmental conditions affect the expression of the volatile compounds, genetics still has the greater contribution. Discriminating compounds among the fragrant varieties included flavor compounds with low odour thresholds, identified for the first time in rice. A model was created in order to determine the combination of compounds relating to each of the sensory descriptors. Along with the flavour compounds, we detected fragrant amine heterocycles which are precursors or products of the fragrance pathway. These compounds provided clues on the chemistry of 2AP synthesis (2-acetyl-1-pyrroline) the major aroma compound in rice. Contrary to previously published works, we propose here an acetylation mechanism of 2AP from its precursor, 4-aminobutanal. SNP mining of the GBS data of the SEA varieties resulted to a total of 69, 033 SNPs widely distributed among the 12 chromosomes. These were used to calculate the similarity indices between the varieties. Results showed very small genetic diversity among some of the SEA varieties, indicating a recent selection process from a similar ancestor, or that active seed exchanges between farmers in the countries of the Mekong Delta Region occurred before the existence of strict import and export regulations. These results together with the metabolomic and sensory data provided a whole picture in the understanding of the traits of aromatic rice which would be beneficial to plant breeders and rice consumers alike. The rice varieties were used to develop a mapping population for subsequent mapping of genetic regions associated with the flavour compounds presented in this study. newly identified flavour compounds, proposed acetylation mechanism of 2AP, genetic diversity of SEA aromatic rice.

POSTER 083

The Bovine Milk Metabolome

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Milk is often called the “perfect food”. No other single food source is capable of growing and sustaining an individual for weeks, months or even years. Not only does milk play a key role in nourishment and hydration, it also plays a key role in establishing essential gut microflora and in priming the immune system in all newborn mammals. Key to its success as food source is its unique, nutrient composition. While many milk composition studies have been conducted, no study has yet attempted to comprehensively profile (identify and quantify) bovine milk using multiple analytical techniques. Here we present comprehensive characterization of the bovine milk metabolome using experimental techniques and computer-aided literature mining tools. Four different types of milk (skim, 1%, 2%, and whole) were analyzed in this study. Nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), direct flow injection-MS/MS (DFI-MS/MS), inductively coupled plasma-MS (ICPOSTER MS), and high performance liquid chromatography (HPLC) techniques were used. A literature review of known metabolites and metabolite concentrations in milk was conducted using several computational text-mining tools, which were originally developed for the Human Metabolome Database. This information was assembled in the Bovine Metabolome Database. More than 470 metabolites were identified and quantified using both experimental and text-mining approaches. A total of 39 metabolites were identified and quantified by NMR, 116 by DFI/LC-MS/MS, 32 by ICPOSTER MS, 12 by HPLC

UV/FD, 20 by GC-MS, and 255 from the literature. All of this information along with other bovine metabolome data is publicly accessible via the Bovine Metabolome Database (BMDB; http://www.cowmetdb.ca/). Metabolite concentrations, structures, related literature references, and other descriptive data are also included. We believe this will serve as a useful source for both metabolomics researchers and dairy scientists by providing a centralized web-based source on the bovine milk metabolome and its unique chemical constituents. This is the most comprehensive and most quantitative metabolomic study of bovine milk to date

POSTER 084

Application of metabolomics to measure the "Foodome"

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Food is fundamental to life. It is the source of essentially all chemical and biological components found in our bodies. As producers, consumers and medical professionals become more aware of the ingredients in foods, there is a growing desire for improved understanding of the precise chemical composition of produced, prepared and consumed foods. Food-derived compounds also provide important information for nutritionists, chemists, and clinicians. The average food item typically contains >10,000 different organic compounds or micronutrients. Unfortunately, the chemical composition of most foods is not well known. Here we present comprehensive metabolomic profiling of a selection of 40 raw foods commonly grown in Canada, including meat, poultry, grains, fruits and vegetables. Nuclear magnetic resonance (NMR) spectroscopy, gas chromatography-mass spectrometry (GC-MS), direct flow injection-MS/MS (DFI-MS/MS), inductively coupled plasma-MS (ICPOSTER MS), and high performance liquid chromatography (HPLC) techniques were combined in order to comprehensively profile the metabolome of 40 different foods. Sample preparation and extraction techniques were developed or optimized for identification and quantification of amino acids, organic acids, phytochemicals, trace metals, sugars, sterols, vitamins, biogenic amines, fatty acids, organic bases, thiols, cofactors and lipids. A literature review of known metabolites and metabolite concentrations was conducted manually and using several computational text-mining tools, including PolySearch and DataWranger, to collect literature data from the Danish food composition database, United States Dept. Agriculture Databases(USDA), Dr. DUKE's pytochemical and ethnobotanical databases, KNAPSACK, and Phenol Explorer. A combination of MS, NMR and HPLC-based techniques allowed us to identify and quantify several hundred metabolites in each sample. Typically NMR assays yielded data on 30-60 compounds per sample. The GC-MS generated data for about 30-75 non-volatile and 20-40 volatile compounds per sample. DFI-MS assays generated data for between 100-180 compounds per sample. ICPOSTER MS assays yielded data on 25-60 metal ions per sample. The MS-based lipidomic and fatty acid assays yielded about 300-600 fatty acids, lipids or lipid species. The specialized HPLC-UV assays yielded more than 20 polyphenols, xanthines and chlorophylls while the vitamin assays generated data for 9 vitamins (B1, B2, B3, B5, B6, B7, B9, B12, and C). Additionally, several specialized assays were conducted that allowed us to measure nucleotides (up to 15 different types) and thiols (7 different compounds). The data has been made publicly available in through the Alberta Food Composition Database (www.afcdb.ca). Each chemical entry in the AFCDB contains more than 100 separate data fields covering detailed compositional, biochemical and physiological information (obtained from the literature). This includes data on the compound's nomenclature, its description, information on its structure, chemical class, physico-chemical data, food source(s), color, aroma, taste, physiological effect, presumptive health effects and concentrations in various foods. Currently, the AFCDB contains more than 40 foods and over 2500

metabolites, and more than 3,000 experimental concentration values of metabolites, some of which have been identified, quantified and reported for the very first time. This represents the first application of comprehensive, quantitative metabolomics to characterizing the food composition of common Canadian foods.

POSTER 085

Linking peptide profiles to phenotypes in dairy products

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Proteolysis is a process where proteins are degraded to peptides. This metabolic process is very important both for the fermentation characteristics in production of yoghurt and cheese and for flavor development (bitter peptides, flavor peptides). Protein degradation is caused by proteases which are either naturally present in milk, added as rennet, or as part of the starter culture. Better understanding of the proteolytic system in the strains used for production will improve the formulation of cultures with regard to production of bitter peptides and control of acidification. The analysis is based on an optimized LC-MS/MS method, acquiring HRMS data in automated data-dependent mode (Agilent 6540 QTOF). Data is converted and loaded into R, where the developed 'pepmaps' package does peptide identification, feature extraction, retention time correction, feature grouping, and matching of features to potential candidate peptides from generated database based on their fragmentation pattern. The 'pepmaps' workflow has been developed at Chr. Hansen and implemented as a routine analysis for profiling cheese, yoghurts, and for substrate analysis. Due to the flexible structure of the 'pepmaps' package, the types of identifications can easily be expanded to incorporate new target peptides including various modifications, provided that it is possible to identify these compounds based on LC-MS/MS data. The current poster will provide examples of this method to different dairy applications. Current challenges lie in improving the absolute quantitative nature of the method. Pepmaps provides a framework for extracting and analyzing LC-MS/MS data from complex samples with focus on peptide identification and quantification.

POSTER 086

Learning from multi compound analyses on yoghurt

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Yoghurt is one of the most popular fermented dairy products and its consumption is increasing worldwide. Therefore, manufacturers are continuously investigating cultures to entice health-conscious consumers. During fermentation, these bacteria perform major biochemical conversions of milk components1 leading to the production of various metabolites resulting in pH decrease, formation of semi-solid texture and distinctive yoghurt flavor. Metabolomics is an effective tool to investigate the overall chemical composition of complex biological systems including matrices as fermented dairy products to achieve metabolome data representing the physiological status of a microorganism. The development a generic analytical method fulfilling these requirements is very challenging, especially in view of the wide range of compound classes, large range of metabolite concentrations present and other physic-chemical conditions. In this project, different yoghurt cultures are investigated in terms of microbial growth, acidification profile and changes in the biochemical composition of milk during yoghurt fermentation for a total of 69 cultures. A complementary metabolomics approach was applied for global characterization of volatile and non-volatile metabolite profiles (such as small acids, organic acids, mono and di-saccharides, amino acids, peptides) of yoghurt associated with activity of the individual strains in the starter cultures. The main analytical techniques are based on HS-GC-FID, GC-TOF-MS and LC-qTOF methods. Sensory evalutaion is performed by trained assessors using quantitative descriptive sensory profiling. Principal Component Analysis (PCA) is performed using MATLAB v. 7.12 (The MathWorks AS, MA, USA) and the PLS-Toolbox

v.7.0.2 software (Eigenvector Research Inc., USA). An evaluation of the data is performed in order to evaluate the reproducibility of chemical analysis, acidification profile and sensory analysis. Preliminary studies suggest differences between some cultures and the chemical profiles. However, more studies need to be done in order to identify effects of the fermentation substrates on the metabolite profiles and avoid masking caused by the differences between the individual bacterial communities. Furthermore, multivariate statistical using Principal Component Analysis (PCA) allows discriminating set and stirred yoghurts fermented by different types of starter cultures according to their metabolite profiles and their physic-chemical characteristic (from sensory panel, pH measurement, cell count). Despite larger variation in the sensory evaluation, previous studies showed that the variations in protein/sucrose content in milk base formulation can be linked to changes in the sensory attributes. This project provides new insights regarding the impact of suitable strain combinations in yoghurt for achieving the best technological performance.

POSTER 087

Metabolic adaptation of hypertrophic muscle in response to extreme dietary challenges and genotypic modifications.

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Myostatin is a powerful regulator of muscle mass growth and metabolism. Deletion of the myostatin gene results in a hyper-muscular phenotype accompanied by compromised muscle function. These mice have a glycolytic phenotype, uPoster regulated anabolic mechanisms and also Impaired mitochondrial contents. The aims of this study was to determine basal metabolic profiles of wild type and myostatin null (Mstn-/-) mice and examine the impact of different dietary regimes on these profiles. In addition, the potential ability of increased expression of the Estrogen-related receptor γ (Err γ) towards a more oxidative metabolism and improved mitochondrial function, in Mstn-/- animals was evaluated. Wild type and Mstn-/- male mice were administered a high-fat diet for 10 weeks or subjected to acute starvation for 24 hours. Mice in the control groups had ad libitum food access. ERRγ transgenic Mstn -/- mice were generated using standard procedures and were maintained on 12-h light/dark cycle and ad libitum food access. 1H NMR spectra were obtained from the aqueous extracts of the gastrocnemius muscle for all samples using a 700MHz Bruker NMR spectrometer. PCA models were built to compare the differences in the metabolic profiles of the groups Hypertrophic muscle was found to use carbon from nutrient sources for the production of biomass rather than ATP production for maintenance purposes. Our results indicate that the metabolic function of muscle from hypertrophic mice was normalised following starvation with their biochemical phenotype resembling that of wild-type animals. In response to a high fat diet, wild-type muscle undergoes metabolic adaptations consistent with its oxidative characteristics, with reductions in tissue lactate reflecting the preferential consumption of fatty acids as a primary energy source. Myostatin null mice do not possess the metabolic flexibility to use dietary fatty acids since they are not taken up by the muscle and cannot be oxidised to sustain the metabolic requirements of the animal. This leads to extreme fat deposition and lipotoxicity detrimental to the survival of the animals. Results from Errγ transgenic mice, with myostatin deletion are still under analysis. Preliminary data from the metabolic signatures of these animals suggest a shift towards a more oxidative phenotype and this is in accordance with results from analyses at the transcript and protein level. This mouse model is an attractive tool for studying muscle wastage in a variety of clinical settings including cancer.

POSTER 088

Top-down targeted metabolomics unearths a new sulfur-containing metabolite with angiotensin-converting enzyme inhibiting activity in Asparagus officinalis

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Asparagus officinalis is a relative species of Allium plants having a variety of S-metabolites. It is shown

that Asparagus has positive effect on reducing systolic blood pressure of spontaneously hypertensive rats. Several reports reveal that nicotianamine and 2''-hydroxynicotianamine are possible inhibitors of angiotensin-converting enzyme (ACE) which catalyzes the reaction from angiotensin I to angiotensin II in the rennin–angiotensin system; however, the existence of other molecules with the ACE inhibitory activity has been suggested so far. Recently, we developed a targeted analysis of S-metabolites (S-omics) using liquid chromatography-Fourier transform ion cyclotron resonance-mass spectrometry (LC-FTICR-MS)1. S-omics was applied to discover new ACE inhibitor in Asparagus. Reference 1. Nakabayashi et al., Anal Chem. 85, 1310 (2013) Given that exact mass and natural abundance on isotopes are reflected on MS spectrum, the theoretical differences in the exact mass and relative signal intensity between 32S-containing monoisotopic ions and their 34S-substituted counterparts can be used for peak-picking of S-ions from metabolome data. Because clear separation of monoisotopic ions and their isotopic ions at the region of the monoisotope (M) + 2 requires high mass accuracy (<1 mDa) and peak resolution (~250,000 full width at half-maximum), the LC-FTICR-MS system (LC, Agilent 1200 series; MS, Bruker Daltonics solariX 7.0 T) that is one of best instrument to obtain ultrahigh resolution data was used to screen S-ions in 47 plant samples, including Asparagus. We obtained 13 S-ions with redundant relative signal intensity. Tandem MS analysis chemically assigned eight S-ions from known S-metabolites such as glucosinolates, glutathione derivatives, and S-alkenylcycteines in Arabidopsis thaliana, onion, garlic, Glycine max, and Wasabia japonica. However, the structure of three ions in Asparagus could not be assigned in this analysis. A new compound asparaptine was therefore isolated using general chromatographic approaches, and its structure was elucidated by MS and nuclear magnetic resonance analyses. Asparaptine was next assayed to evaluate its inhibitory activity against angiotensin-converting enzyme (ACE) along with positive markers such as captopril, N-succinyl-L-proline, and nicotianamine. The IC50 values of captopril, nicotianamine, N-succinyl-L-proline, and asparaptine, were 1.61 nM, 18.7 μM, 14.5 μM, and 113 μM, respectively. The amount of asparaptine was 251 mg/kg FW which is approximately 5-fold more than 2"-hydroxynicotianamine, whose inhibitory activity is nearly equal to nicotianamine in Asparagus. Asparaptine was identified as a new S-containing ACE inhibitor in Asparagus.

POSTER 089

Metabolic profiling of pancreatic β-cells reveals a mechanism for the protective effects of a milk-derived bioactive

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Type 2 diabetes mellitus (T2DM) is a metabolic disorder of glucose homoeostasis characterized by hyperglycemia, insulin resistance and pancreatic β-cell dysfunction. Glucotoxicity causes β-cell deterioration, decreases insulin secretion, leads to β-cell dysfunction which is irreversible over time. Nutritional strategies for protecting β-cells against glucotoxicity are of high interest. Application of metabolomics to study β-cell metabolism offers a unique insight into the metabolic functionality of these cells. The objectives of this study were (1) to examine the ability of a milk-derived bioactives to protect β-cells from glucotoxicity and (2) to determine the underlying mechanism of such protection using metabolomics. In this study, INS-1E and BRIN-BD11 β-cells were treated with 25 mM glucose (HG) to mimic glucotoxicity or 25 mM glucose plus 1 mg/mL milk-derived bioactive (HG + bioactive), in parallel with 11.1 mM glucose as control for 20 h. Following 20 h exposure, insulin secretion, metabolic profiling and functional assays such as plasma membrane potential (PMP) were performed. Fatty acids and amino acids were analyzed using metabolomics platforms GC/MS and NMR, respectively. The metabolites from NMR peaks were identified and quantified using Chenomx NMR Suite. Multivariate data analysis was performed with SIMCA-P+12. Statistical analysis was performed using SPSS 20 using one-way ANOVA with LSD as post-hoc test at the significant level of $P < 0.05$. Prolonged exposure to HG reduced glucose stimulated insulin secretion in INS-1E cells from 701.6 ng/mg protein to 308.7 ng/mg protein ($P = 3.17 \times 10^{-12}$). Addition of the milk-derived bioactive restored insulin secretion to 554.1 ng/mg protein ($P = 4.20 \times 10^{-4}$). A similar result was also observed in the second cell line, thus validating the protective effect of the milk-derived bioactive. Robust PLS-DA plots from NMR data demonstrated clear separations both between control and HG groups (R2X: 0.635; Q2: 0.885) and between control and HG+ bioactive groups

(R2X: 0.647; Q2: 0.940). Ten metabolites were identified from the variable importance for the projection (VIP) > 2. The metabolites glutamate, aspartate, o-phosphocholine, glycero-phosphocholine and taurine showed the strongest discriminatory signals in control group in both models, while alanine, GABA (4-aminobutyrate), creatine and glucose strongly discriminated in the HG group or in the HG+bioactive group. Metabolomic analysis revealed that prolonged exposure to HG decreased the levels of C16:1, C20:4n6, C20:2n6, C22:6n3, total PUFAs and aspartate significantly, and increased GABA, alanine and creatine significantly. Addition of the milk-derived bioactive restored the concentrations of C16:1, C20:4n6, C22:6n3, total PUFAs and aspartate significantly towards control conditions and also increased the concentrations of C22:1n9, and GABA significantly in comparison with control group. Furthermore, prolonged exposure to HG decreased the response of plasma membrane potential to glucose stimulation significantly ($P = 0.014$). Addition of the milk-derived bioactive restored the plasma membrane potential towards control conditions ($P > 0.05$). Overall, the present results demonstrate that the milk-derived bioactive protected the β-cells from dysfunction under glucotoxic conditions in terms of insulin secretion. The identified alteration in the fatty acids, amino acids and plasma membrane potential represents a potential mechanism by which the milk-derived bioactive protects the β-cells against glucotoxicity. Metabolomics identified altered pathways which may explain protective effects of milk-derived bioactives.

POSTER 090

From the Exposome to the Metabolome: Profiling Tomato Juices and Their Effects on the Blood Metabolome

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The consumption of tomato products has been associated with a decreased risk of chronic diseases and carotenoids are believed to be partially responsible for this protective effect. The tangerine tomato has a unique carotenoid profile compared to the traditional red tomato, which translates into a difference in bioavailability and potentially in biological effects. The objective of this work was to profile phytochemicals in red and tangerine tomato juices intended for clinical interventions and to determine the effects of these tomato varieties on the blood metabolome of mice. Untargeted metabolomic analyses were conducted on the tomato juices and blood samples using an Agilent iFunnel 6550 QTOF-MS with a 1290 Infinity UHPLC. Red and tangerine tomatoes grown and processed into juices at the Ohio State University were analyzed for polar and non-polar phytochemicals using ESI and APCI ionization modes, respectively. Blood collected from mice fed a control, red tomato, or tangerine tomato diet for four weeks was analyzed for polar metabolites. Pooled QC samples were used to monitor data quality and platform stability. Data from both experiments were processed and analyzed using Agilent Profinder and Mass Profiler Professional software. Compounds were identified using accurate mass, MS/MS fragmentation patterns, and authentic standards when available. The red and tangerine tomato juices were found to differ significantly in a number of phytochemicals, including carotenoids, chlorophylls, catecholamines, dihydrochalcones, hydroxycinnamic acids, and polar lipids. Many of these compounds have been shown to possess biological activity and may contribute to the protective effect of tomato products. These tomato varieties were also found to significantly alter over 100 blood metabolites in mice over a 4 week period. Datasets are currently being mined to identify metabolites and to determine if the red and tangerine tomatoes have a differential impact on the blood metabolome. An untargeted metabolomics approach was applied to determine changes in the blood metabolome of mice fed unique tomato varieties.

POSTER 091

Impact of food processing on whole grain rye and wheat phytochemicals and their bioavailability as examined with non-targeted metabolomics approach

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Whole grain products are an essential part of healthy diet as their high consumption has been linked epidemiologically to reduced risk of various chronic diseases including type 2 diabetes, cardiovascular diseases and certain cancers. The indication of the precise constituents responsible for biologically relevant, health protective metabolic effect of whole grains is difficult e.g. due to the large number of phytochemicals, including hitherto unknown compounds, present in the bran compartment. Furthermore, different processing methods during the food manufacturing modify quantitatively and qualitatively the phytochemical composition of the product resulting in different bioavailability of compounds and ultimately may impact the biological outcome. We have analyzed phytochemicals present in whole grains and investigated how differentially processed rye and wheat bran fractions exert phytochemicals in mouse feeding trials as well as in human dietary studies. A comprehensive mouse feeding experiment with bioprocessed fractions of rye bran and wheat aleurone was carried out and non-targeted metabolite profiling utilizing combination of RP and hilic chromatography with qTOF-MS analysis has been applied on biofluids and samples from internal organs. The pattern of metabolites excreted in urine varied depending on the grain species and the bioprocessing method applied. Similarly, occurrence of phytochemical metabolites in plasma was differential, and interestingly, their accumulation pattern in various internal organs was also differential according to the feed. The phytochemical metabolites in organs are anticipated to potentially participate in cellular metabolism and cause fine tuning of metabolism eventually evidenced as improved metabolic health. This is indicated by modulation of the endogenous metabolite homeostasis in the intestinal organs e.g. liver and heart. We have observed that bioprocessing affects the intestinal metabolism of several phytochemical classes including phenolic compounds and benzoxazinoids potentially via changed bioaccessibility. Furthermore, our results indicate that glycine betaine, which is abundant in both rye and wheat bran, is metabolized potentially via metabolism of microbiota resulting in urinary excretion of several amino acid derived betaines. The observations made with the mouse studies are presently evaluated with results obtained from human intervention trials to find out whether any similar metabolic phenomena is found. Profiling of biofluids and organs enables gaining wider view of metabolic effect of diet than samples from human studies solely

POSTER 092

In vitro and in vivo metabolic fate of black tea polyphenols

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Black tea has been associated health benefits such as reduced risks of cardiovascular diseases and cancer. These health benefits are hypothesized to be mostly attributed to polyphenols, the major constituents of black tea. Yet, it is still unclear which of these polyphenols exert bioactivity, partly because polyphenols are extensively metabolized and a comprehensive view on the metabolites formed is still lacking. In particular, gut microbiota play an essential role in the metabolism of black tea polyphenols. They degrade diverse polyphenols such as catechins, thearubigens and theaflavins to smaller phenolics that can be further absorbed by the host. In addition, polyphenols and their metabolites undergo phase II metabolism such as methylation, glucuronidation and sulphation. We aimed at identifying metabolites

from black tea polyphenols using both in vitro and in vivo studies. Different state-of-the-art profiling techniques were applied to capture the vast diversity of metabolites produced from black tea. Initially, we explored the inter-individual variation in the gut microbial bioconversion of black tea extract from 10 healthy human subjects using in vitro fecal batch fermentations. We found that the complex polyphenol mixture was degraded to a limited number of key metabolites. Each subject displayed a specific metabolite profile differing in composition, concentrations and time courses. Furthermore, we designed a single- and continuous-dose experiment using a five-stage in vitro gastrointestinal model (TWINSHIME) to get more insight into the metabolism occurring in the different colon compartments. Clear differences in the metabolites produced were observed between single- and continuous dosing and between the compartments. Last but not least, we performed a randomized, open, placebo-controlled, cross-over study, in which twelve healthy men consumed a single bolus of black tea extract or a placebo. In total, 58 conjugated metabolites of black tea polyphenols were identified from plasma collected at several intervals over a period of 30 hours. The kinetic profiles revealed that conjugated catechins appeared first in plasma (0.5 h), followed by diverse conjugated microbial metabolites (1-2 h). The cmax values differed by two orders of magnitude between directly absorbed catechins and gut microbial bioconversion products. Potential leads were identified for further bioactivity testing and for elucidating the mechanisms-of-actions underlying the health benefits of black tea.

POSTER 094

Protective effect of agaro-oligosaccharides on high-fat diet-induced intestinal dysbiosis in mice: correlation between microbiota and bile acid profile

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Alterations in the composition of gut microbiota, known as dysbiosis, are caused by several factors including dietary components and environmental factors. In particular, high-fat diet (HFD)-induced dysbiosis is increasingly recognized as a major risk factor for various diseases such as colon cancer and diabetes. Agarose, which is the predominant component of polysaccharides in agar, is hydrolyzed easily to yield oligosaccharides. The resultant oligosaccharides termed agaro-oligosaccharides (AGOs) (Agaphytose TM) possess various physiological activities. However, the effect of AGOs on gut microbiota remains unknown. In this study, we investigated the effect of AGOs on HFD-induced intestinal dysbiosis in mice, particularly in correlation between microbiota and bile acid profile. C57BL/6N mice were fed standard diet (SD) with 4.6% of calories from fat, HFD with 32% of calories from fat, and with or without AGOs for 8 weeks. AGOs was supplemented in the drinking water as a concentration of 3.0% (w/w) and administered to the SD+AGOs and HFD+AGOs groups. To analyze the fecal microbiota profiles, we performed terminal restriction fragment length polymorphism (T-RFLP) approach. The profiles of cecal organic acids and serum bile acids were determined using HPLC and LC-MS/MS systems, respectively. T-RFLP analyses showed that HFD induced a significant change in the gut microbiota. The changes in the microbiota composition induced by HFD were characterized by the decrease in the order Lactobacillales, and the increase in the genus Bacteroides and Clostridium subcluster XIVa. These changes of the microbiota community generated by HFD treatment was significantly suppressed by AGOs supplementation. Next, we determined the concentrations of cecal organic acids as metabolites of microbiota. The concentrations of acetic, propionic, and butyric acid decreased in HFD-received mice, and these down-regulation of organic acid concentrations were unaffected by AGOs supplementation. The concentration of lactic acid significantly increased in HFD+AGOs group. Furthermore, to investigate

the effect of the change of gut microbiota community on bile acid metabolism, we evaluated the serum bile acid profile. We determined four primary bile acids (cholic, chenodeoxycholic, a-muricholic, and b-muricholic acid) and three secondary bile acids (deoxycholic, ursodeoxycholic, and lithocholic acid) and their glycine and taurine conjugates. The data from serum bile acid profile showed that the level of deoxycholic acid (DCA), a carcinogenic secondary bile acid produced by gut bacteria such as strains belonging to Clostridium subcluster XIVa, was increased in HFD-received mice. The uPoster regulation of DCA level generated by HFD treatment was suppressed by AGOs supplementation. With regard to the correlation between gut microbiota and bile acid profile, AGOs supplementation prevents HFD-induced intestinal dysbiosis.

POSTER 095

Plasma and urinary metabolomics in assessing treatment responses to protein supplementation via an accelerated workflow using multiplexed separations on CE-QTOF-MS

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Protein supplementation has been shown in recent studies to attenuate the loss of muscle mass during weight-loss interventions. However, the mechanism by which increased protein consumption enables the loss of fat mass while retaining muscle mass is unknown. In order to gain insight into the metabolic processes associated with protein supplementation, as well as differences in gut microbiota metabolism following a 2-week intervention of whey or soy protein, plasma and urine samples of 40 healthy overweight/obese adults are analyzed by an accelerated data workflow using capillary electrophoresis multi-segmented-injection quadrupole-time-of-flight mass spectrometry (CE-MSI-QTOF-MS). 40 healthy overweight/obese adults (male: n = 19; female: n = 21), aged 35-65 years, with BMI in the range of 28-50 kg/m2, were randomly assigned to receive twice-daily supplements of isolated whey protein, soy protein or maltodextrin (control) as part of a controlled hypoenergetic diet over a 14-day period. Blood samples and morning urine samples were collected before and after the dietary intervention for analysis by capillary electrophoresis quadrupole-time-of-flight mass spectrometry (CE-QTOF-MS). Preliminary analyses of pooled samples of the whey, soy and control groups, respectively, have revealed the whey subgroup exhibiting a 3-fold increase in the post-prandial urinary level of taurine relative to baseline, while the soy and control groups exhibit little to no change in urinary taurine levels. This observation suggests the possibility of a significant underlying perturbation in sulfur metabolism brought on by the increased consumption of whey protein. Other related sulfur metabolites will be investigated in the pre- and post-prandial plasma and urine samples in order to shed light on sulfur metabolism and the mechanism of whey protein in particular on muscle maintenance during weight loss. Accelerated data workflow for biomarker discovery via multiplexed separations

POSTER 096

Discovery-Based High-Resolution Plasma Metabolomics Following a Vitamin D Intervention in Adult Patients with Cystic Fibrosis

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Cystic fibrosis (CF) is a life-threatening disease affecting multiple organ systems, with clinical outcomes strongly influenced by nutritional and metabolic status. Advances in metabolomics provide a novel opportunity to broadly explore metabolism and underlying pathways relevant to nutrition and disease progression in adults with CF. Pilot studies suggest that vitamin D3 supplementation reduces inflammation and improves clinical outcomes in adults with CF; however, the metabolic effects of vitamin D are little understood. This study aimed to explore the impact of high-dose vitamin D3 supplementation on metabolism during acute illness using high-resolution plasma metabolomics analysis. A secondary aim was to explore additional nutrition-related metabolic pathways that were associated with clinical variables (lung function, length of hospital stay) during acute illness. Subjects were 25 adult CF patients hospitalized for acute pulmonary exacerbation, randomized to receive an oral bolus of 250,000 IU vitamin D3 or placebo. EDTA plasma was obtained before study drug dosing, and 7 days and 3 months after hospitalization. Acetonitrile-treated samples were spiked with internal standards and analyzed in triplicate using high-resolution liquid chromatography-mass spectrometry (LTQ-Velos Orbitrap) with positive ESI on a C18 column. apLCMS and xMSanalyzer were used for peak detection, alignment, and generation of a m/z feature table with associated retention times and intensity values. LIMMA, linear regression, repeated measures ANOVA, false discovery rate (FDR), and hierarchal clustering analysis (HCA) were conducted. Putative metabolite identification and pathway analyses were performed using METLIN and Mummichog pathway software, respectively. Over 11,000 m/z features were detected. A cross-sectional analysis using LIMMA to compare the vitamin D and placebo groups 3 months after hospitalization detected 12 metabolites differing between groups after FDR (q = 0.20) correction. HCA showed clear separation between subjects receiving vitamin D3 and those receiving placebo at month 3. Putatively identified differentiating metabolites included a vitamin D3 derivative that was higher in the vitamin D-treated group, and three ceramide-related complex lipids (including gangliosides) that were lower in the vitamin D-treated group. Within the vitamin D3-treated group, 13 metabolites differed between baseline (acute exacerbation, pre-study drug treatment) and 3 months (FDR q = 0.20), including several metabolites related to the methionine/cysteine cycle and membrane lipids. Two-way repeated measures ANOVA also indicated group differences in a ganglioside and S-adenosylhomocysteine. Thus, administration of vitamin D3 appeared to influence several pathways, including those involved in lipid membrane and methionine/cysteine metabolism, which are known to be altered in CF. Metabolome-wide association studies of lung function [expressed as a % of the predicted forced expiratory volume in 1 second (FEV1%)] and length of hospital stay, incorporating linear regression in combination with Mummichog pathway analysis, identified several significantly associated nutrition-related pathways: FEV1% was associated with 25 pathways including folate, omega-3 fatty acid, and glutathione metabolism. Hospital length of stay was associated with 27 pathways, including several carbohydrate and amino acid metabolism pathways. Relevant to our vitamin D analyses, both FEV1% and length of stay were associated with methionine/cysteine metabolism and glycosphingolipid biosynthesis pathways, suggesting lung function may be linked to hospital outcomes in this cohort by similar metabolic pathways. Taken together, these data may inform targeted investigation of nutrition-related biomarkers and interventions during acute pulmonary illness in adult CF, although metabolite verification and confirmation with larger samples sizes is needed. These data provide insight into potential metabolic effects of vitamin D3 and nutrition-related pathways during acute pulmonary exacerbation in CF.

POSTER 098

Sodium nitrite supplementation modifies the plasma metabolome in older adults: relation to physiological function

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Physiological dysfunction occurs with advancing age and increases the risk for clinical disease and

disability. Reduced bioavailability of the vaso-active and -protective molecule nitric oxide (NO) contributes to vascular dysfunction, which is linked to reduced motor and cognitive function. Sodium nitrite (SN) supplementation increases circulating nitrite, a precursor of NO, and improves select domains of physiological function in older animals and humans. Although mechanisms underlying these changes are complex, the plasma metabolome may reflect alterations to metabolic pathways contributing to dysfunction. We hypothesized that SN will improve vascular, motor and cognitive function in middle-aged and older adults (MA/O), and that the plasma metabolome will offer mechanistic and predictive insight into pathways altered with SN treatment. Healthy MA/O adults (62 ± 1 years) received 10-weeks oral SN (80-160 mg/day, TheraVasc, Inc.; n=20) or placebo (n=10). Vascular endothelial (brachial artery flow-mediated dilation [FMD]), motor (knee-extension [KErtd]; knee–flexion [KFrtd]) and cognitive (trail-making test B [TMTb]) function was assessed pre/post. EDTA-treated plasma was collected pre/post and frozen at -80C until analysis. Samples were prepared for untargeted metabolomics using combined protein precipitation/liquid-liquid extraction and separated into lipid/aqueous fractions. LC-MS (Agilent 6410) spectral data were extracted using Mass Hunter Qualitative Analysis (Agilent). Molecular features were annotated in IDBrowser (Agilent) using in-house databases. Metabolites significantly altered by SN were confirmed using MS/MS (Agilent 6520) and matched to the NIST 14 library. Regressions were employed to identify molecules associated with physiological function. 10 weeks of SN treatment increased plasma nitrite concentrations 10 to 20-fold above baseline and improved post-intervention measures of brachial FMD (5.7 ± 0.6 vs 3.7 ± 0.4, $p<0.05$), KErtd (1206 ± 449 vs 933 ± 330, $p<0.05$), KFrtd (1394 ± 411 vs 1009 ± 281, $p<0.05$), and TMTb (48 ± 11 vs 57 ± 15, $p<0.05$) compared to pre-treatment values, independent of changes in clinical parameters or lipid profiles. No changes in plasma nitrite concentration or improvements in physiological function were observed with placebo treatment. Untargeted metabolomics analysis revealed significant changes with SN supplementation in 75 of the ~4,000 detectable metabolites compared to placebo. Importantly, identified metabolites resided in pathways previously associated with a pro-inflammatory and pro-aging phenotype, including metabolites of glycerophospholipid, sphingolipid, fatty acid ester, amino acid, purine, pyrimidine, and peptide metabolism. After application of the Benjamini-Hochberg procedure, 22 metabolites remained significant and were analyzed in subsequent regression models. In these analyses, specific metabolites altered with SN were identified as being significantly correlated with improvements in vascular and cognitive function. Moreover, baseline concentrations of metabolites altered by SN were able to explain a portion of the variance in measures of baseline vascular, motor and cognitive function. In addition, these baseline metabolite concentrations were also able to significantly discriminate between individuals for responsiveness to SN in measures of vascular and combined motor/cognitive function. These results indicate that SN modulates multiple metabolic pathways in healthy MA/O adults, and these modifications are associated with improvements in physiological function. Most importantly, these data illustrate the ability of the plasma metabolome to predict measures in multiple domains of physiological function and forecast an individual's responsiveness to intervention. In older adults, the plasma metabolome predicts physiological function at baseline and improvements in response to sodium nitrite supplementation.

POSTER 099

Comparing Metabolite Profiles of Habitual Diet in Serum and Urine

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Diet plays an important role in chronic disease etiology, but some associations remain inconclusive due to methodological limitations in measuring dietary intake. Better dietary assessments are needed to accurately estimate the strength of diet-disease relationships. Metabolomics offers a novel method for identifying objective diet biomarkers, although it is unclear how diet-metabolite correlations measured

from serum compare with those from urine. We estimated correlations between intake of 56 food, beverage, and dietary supplement items assessed by food frequency questionnaire (FFQ), with 415 serum and 374 urine metabolites identified by untargeted liquid chromatography/mass spectrometry (LC-MS) and gas chromatography/mass spectroscopy (GC-MS) in the Navy Adenoma Study (N=125 cases, 128 controls), adjusting for age, sex, smoking, fasting, and case-control status, body mass index, and daily caloric intake. We used 10-fold cross validation Least Absolute Shrinkage and Selection Operator (LASSO) regression to construct metabolite linear models for prediction of self-reported dietary intake. We compared correlations between diet intake predicted by the metabolite profiles and self-reported diet to assess the quality of metabolomics in serum and urine to capture self-reported diet. In our sample of predominantly male (77%), non-smoking (93%) participants with mean age 57±9 years, we identified metabolites associated with 39 of 56 diet items (food, beverages, and supplements). In urine, 203 metabolites (range r=-0.3 to 0.5, p less than 9.6E-20 to 1.0E-03) and in serum 52 metabolites (range r= -0.4 to 0.6, p less than 6.3E-19 to 1.1E-04) were associated with at least one dietary item after control for multiple comparisons at FDR less than 0.1. Of these, 38 urine and 10 serum metabolites associated with multiple diet components. Twenty-eight diet-metabolite correlations, the majority with r above 0.3, were common between serum and urine measurements. We replicated previously reported findings for most diet items, for example stachydrine as a biomarker for citrus intake (r=0.53). Previously unreported associations were identified, including with apple (serum indolepropionate [r=0.24], urine threitol [r=0.22] and 3-hydroxyphenylaceate [r=0.21]), citrus (urine N-methylglutamate [r=0.34]), nuts (urine pinitol [r=0.23]), processed meat (urine ectoine [r=0.23]), shellfish (urine ciliatine [r=0.24]), sugar (trimethylamine N-oxide [r=0.21]) and coffee intake (urine nicotinic acid [r=0.3]). Dietary intake predicted from urine metabolite profiles was more strongly correlated with self-reported diet for a greater proportion of tested diet items compared with serum. Sensitivity analyses found no differences in diet-metabolite correlations by case-control status. In summary, metabolite biomarkers of self-reported diet were identifiable in both serum and urine with similar strength of associations, although more metabolites were identified for a given food in urine. Collection of urine samples may offer a valid alternative to serum for diet metabolomics in large-scale epidemiologic studies. We identified several novel dietary metabolite biomarkers. Overall, more urine dietary metabolites were identified compared with serum.

POSTER 100

Dissecting metabolic markers of consumption of functional foods: metabolomic-based characterization of coffee consumption

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Coffee is well known to have bioactive compounds and to be associated with several medical traits. However, reproducible data evidencing the good or bad role of consumption of these foods in health is not a consensus in the literature. Several are the reasons for the lack of consensus. Part of this disagreement is due to variations in the metabolization of these foods between subjects, varying for how long and how much the body is exposed to these bioactive molecules. Given the potential misclassification of consumption, association studies using only consumption as a proxy of exposure are expected to be far from optimal. As a result, data providing evidence for the beneficial role of these foods are still lacking. Here, we aimed to identify differences in coffee metabolization profile with potential use in epidemiological studies. 30 healthy men were recruited, subjected to anthropometrical, nutritional and dietary evaluation. Fasting blood and urine were sampled before coffee ingestion. Following, the participants drank 150mL of Brazilian coffee (Coffea Arabica - Prima Qualitá®) prepared by infusion and filtration. Serum and urine were sampled at 6, 12 and 24 hours after intake. Serum samples were deproteinized, followed by lyophilization and derivatization with MSTFA/TMCS. 1µL of this derivative was

used for GC/MS analysis (Shimadzu GC-MS). SIM acquisition was performed monitoring 194m/z and 109m/z caffeine ion and Scan (monitoring from 50m/z to 500m/z) data was analyzed with NIST 11 compound library. We experimentally characterized metabolization profiles, as well as interindividual variability in the metabolomic response profile after a well-controlled coffee intake in a homogeneus group of study subjects. We identified around 200 metabolites in each sample. Based in caffeine quantification, we identified two patterns of caffeine metabolism: fast and slow. Eighteen subjects showed caffeine until 12 hours after intake and were classified as slow metabolizers, while seven individuals did not show caffeine in the same period, which were grouped and classified as fast metabolizers. In addition to caffeine metabolism, groups showed different metabolic profiles in each monitored time. Some metabolites such as 7-Methyl caprilic acid, Ribose, Gluconolactone, Barbituric acid and Malonic acid were presente just in the Fast Group, whereas Uridine, 11-Eicosenoic acid, Glycolic acid, 2-Aminobutyric acid, Asparagine, Pelargonic acid, Valeric acid, Ethanolamine and Sorbitol were present only in the Slow Group. Despite the differential coffee metabolism, these subjects do not had differences in the consumption of carbohydrates, lipids, fiber, caffeine, iron, calcium, phosphorus, potassium, zinc, selenium, sodium, vitamin C, thiamine, riboflavin, niacin, pyridoxine, folate and the total caloric intake, as evaluated by consumption dairies. There was a difference in protein and cholesterol consumption between groups. New experiments with urine samples are being performed to analyze caffeine excretion between groups. Finally, CYP1A2 polymorphisms are being analyzed. Once characterized, markers of consumption and of downstream metabolic response will be measured in a larger cohort of 1,500 individuals participating in the already established ADVENTO study, an ongoing cohort aiming to study the cardiovascular effects of different diets (omnivorous versus vegetarian) in the Brazilian population. Development and characterizations of new markers of health-related food consumption.

POSTER 101

Metabolomics based discovery of a panel of biomarkers for assessment of red meat intake

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Red meat intake has been associated with increased incidence of several chronic diseases, including cancer, cardiovascular disease and diabetes. However, the association between red meat intake and health risks remains controversial. To improve the classification of a person's dietary intake and therefore clarify proposed links, reliable and accurate dietary assessment methods of food intakes are essential. The identification of novel biomarkers of red meat intake, through the application of metabolomics, offers the potential of a more objective measure of red meat intake. This study aims to identify a panel of specific metabolites that reflect red meat intake using data from the food intervention study Nutritech. 10 NutriTech participants consumed a red meat meal for 3 days during 3 consecutive weeks in a controlled environment in a metabolic suite. Protein in the form of red meat, increased from 13% of energy in week 1 to 30% of energy intake in week 3. Postprandial urine and plasma samples (2, 6 h) were collected on day 3 of each intervention week. Fasting samples were also collected. Plasma samples were analysed by BIOCRATES' TargetIDQTMp180 Kit and specific metabolites were identified and quantified. Urine samples were analysed using 1H nuclear magnetic resonance (1H NMR) spectroscopy. Multivariate data analysis identified discriminatory spectral regions between postprandial urine samples across the weeks. Discriminatory spectral regions were identified and subsequently quantified using Chenomx. Plasma results revealed a significant increase in 1-methylhistidine, 3-methylhistidine and trimethylamine N-oxide (TMAO) in postprandial samples following meat consumption across the 3 intervention weeks. 3-methylhistidine and TMAO also increased significantly in fasting samples across the 3 weeks. However, 1-methylhistidine demonstrated an increasing non-significant trend. Metabolites including trans-4-hydroxyproline, isoleucine, leucine and methioninesulfoxide also increased in postprandial samples in response to increasing red meat intake across the weeks. Trans-4-hydroxyproline, isoleucine and leucine significantly increased in the fasting samples across the 3 weeks. Acylcarnitines C4 and C5 also increased significantly in postprandial samples across the 3 weeks following an increased intake of red meat. C4 increased significantly in the fasting samples across the 3 weeks.Partial least-squares

discriminant analysis (PLS-DA) models revealed that the 6h postprandial sample compared between week 1 and week 3 had the highest Q2 value (0.762). A permutation test confirmed the model's validity (Q2= 0, -0.264). Discriminating metabolites between week 1 and week 3 in the 6h postprandial sample were extracted using an S-line plot generated from an orthogonal PLSDA. 18 metabolites were identified including, 3-methyl-2-oxovalerate, 2-oxoisocaproate, o-acetylcarnitine, tyrosine and carnitine. A number of these metabolites were also discriminating metabolites between low and high red meat intake in fasting samples. Following quantification of each metabolite, quantified metabolites in the 6h postprandial sample were compared across the weeks by repeated measures ANOVA. The metabolites demonstrated an increasing trend from week 1 to week 3 that corresponded to increasing red meat consumption over the 3 weeks. Metabolites including 3-methyl-2-oxovalerate, o-acetylcarnitine, tyrosine and carnitine also increased in fasting samples across the 3 weeks. This work has clearly identified a series of metabolites that respond to increased consumption of red meat. Future work will assess the ability of these biomarkers to discriminate between a range of red meat consumptions in a cross-sectional study. Identification of an objective panel of red meat biomarkers in an intervention study and subsequent validation in a cross-sectional study.

POSTER 102

Metabolomic Screening of Urinary Bioactive Compounds After Acute and Chronic Consumption of Tropical Highland Blackberry (*Rubus adenotrichos*) Juice

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Tropical highland blackberry (Rubus adenotrichos) juice is widely consumed from Costa Rica to Ecuador and represents the main source of anthocyanins and ellagitanins for some population. The screening of metabolites that appears in urinary excretions after consuming the juice aims at evidencing the exposure to specific bioactive phytomicronutrients and their metabolites. This metabolomic approach followed should help to discover the potential of blackberry juice as a functional food with positive impact on the health of consumers. 15 healthy male volunteers were included in a crossover nutritional intervention. For the acute exposure, after ingestion of a placebo or 250 ml of blackberry juice, the urine 0-6 hours, 6-12 hours and 12-24 hours were collected. For the chronic exposure, 24h urine was collected and after 9 days of daily intake of 250mL. Urine samples were analyzed randomly by UPLC/ESI-Q-TOF/MS on positive mode and transformed by MZmine.and analysed by multivariate statistical analysis to assess linear combination of ions responsible of the differences between the control group and the group with blackberry juice. Identification of parent ion was performed thanks the use of standards and/or after comparing fractionation pattern with international database. After chronic intake, urolithin-A (UA) glucuronide, urolithin-B (UB) glucuronide and urolithin-C (UC) aglycone were detected as the main discriminating metabolites after blackberry juice consumption. These compounds resulting from the degradation by microbiota of the ellagitannins are claimed to be active on hormone-dependant cancer cells. Another metabolite, terpinen-4-ol proceeding from the metabolism of blackberry aroma compound was also observed. Additionally, the renal excretion of the endogenous uric acid showed a significant increase which confirms the traditional use of these berries against gout disease. During the acute intervention, it was possible to confirm the apparition of urolithins after 12 hours. In addition to the appearance of metabolites proceeding from the degradation of phenolic compounds such as hippuric acid and chalcone, it was possible to evidence a significant decrease in the excretion of two gluco-corticoides, the tetrahydrocortisone (THE) and tetrahydro-11-dehydrocorticosterone (THA) involved in the cholesterol cycle. The metabolomic approach is proved to be an interesting diagnostic tool to assess the functionality of food

POSTER 104

Effects on Metabolites of Reduced Dietary Sodium and Dietary Patterns: The Dietary Approaches to Stop Hypertension (DASH) -Sodium Feeding Study

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Many nutritional exposures are poorly measured by questionnaires used in epidemiologic studies. Sodium intake is extremely difficult exposure to quantify and is known to affect blood pressure but its relation to other health outcomes is equivocal. The DASH diet is high in fruits, vegetables, and low fat dairy products and corresponds to a “Healthy” dietary pattern. The primary aims of the present study were to identify metabolites which 1) determine high (3,500 mg) and low (1,150 mg) sodium intake and 2) distinguish the DASH diet compared to a control or typical American diet. Such profiles can then be applied to cohort studies that have stored blood samples to evaluate association with diseases and understand mechanisms that underlie associations. We measured metabolites in plasma from 120 DASH-Sodium Feeding Study participants (50% African American/50% Caucasian, and 50% men/50%women). Sixty participants consumed the DASH diet and 60 the control diet (parallel-group design). Within each diet arm, all participants consumed foods low or high in sodium in random order for 30 consecutive days (crossover design). Metabolites were measured by Metabolon Inc. using the Q Exactive MS platform in fasting EDTA plasma samples were collected ate the end of each sodium intervention. We used paired t-tests to evaluate the average effects of sodium intake on metabolites. Linear mixed effect models were used to determine effect of diet on metabolites while controlling for effects of age, gender, race and sodium intake. The top known metabolites that on average significantly differed among those consuming higher compared to lower sodium intakes were as follows (difference (standard error)): 4-ethylphenylsufate (-0.18 (0.04)), pelargonate (-0.06 (0.02)), 1-linoleolglycerophosolipid (-0.19 (0.02)) methionine sulfone (-0.06 (0.01)), 4-hydroxyphenylpyruvate (-0.05 (0.05)), myoinositol (-0.04 (0.01)), asparagine (-0.07 (0.02)), xanthine (-0.10 (0.03)), taurine (-0.09 (0.02)), urate (-0.08 (0.007)), o sulfo-l-tyrosine (-0.02 (0.009)), 1 arachidonoylglycerophospholipid (-0.05 (0.01)), N delta-acetylornithine (-0.10 (0.03)), and 4-allyphenolsulfate (-0.08 (0.03)) were lower while gamma glutamylisoleucine (0.14 (0.04)) and gamma glutamylvaline (0.13 (0.04)) were higher among those consuming higher sodium intake (Poster value <0.0015). 4-ethylphenylsufate was the only metabolite that reached Bonferoni correction significance (Poster value < 10-5). There was evidence that the effect of sodium intake on these metabolites was greater among those consuming the control or typical American diet. The DASH diet compared to the control diet resulted in significantly higher concentrations of methyl-glucopyranoside (difference (95% confidence interval) 0.60 (0.47. 0.72)), stachydrine (1.10 (0.94,0.26)), tryptophan betaine (0.45 (0.32, 0.57)), 2-aminophenolsulfate (0.39 (0.27, 0.50)), pyridoxate (0.18 (0.13, 0.24)), 4-allylphenolsulfate (0.30 (0.20, 0.40)), 4-vinylphenolsulfate (0.36 (0.24, 0.49)), dopamine sulfate (0.25 (0.16, 0.34)), myoinositol (0.11 (0.07, 0.15)), indolepropionate (0.29 (0.18, 0.41)), docosahexaenoate (0.12 (0.07, 0.17)), pantothenate (0.11 (0.06, 0.16)), 2-hydroxydecanoate (0.11 (0.06, 0.16)), pipecolate (0.17 (0.09, 0.25)), uridine (0.06 (0.03, 0.09)), N delta-acetylornithine (0.17 (0.09, 0.25)), urea (0.08 (0.04, 0.11)), and 2-hydroxyibutyrate (0.07 (0.04, 0.10)), and lower concentrations of oleoyl-sphingomyelin (-0.12 (-0.09, -0.15)), gamma tocopherol (-0.29 (-0.19, -0.39)), theombromine (-0.70 (-0.44, -0.96)), 7-methylxanthine (-0.49 (-0.29, -0.69)), trans-4-hydroxyproline (-0.10 (-0.06, -0.14)), S-methylcysteine (-0.11 (-0.07, -0.16)), palmitoleoyl sphingomyelin (-0.05 (-0.03, -0.08)), and indolebutyrate (-0.26 (-0.13, -0.39)). All these metabolites were significant at the Bonferoni correction (Poster value < 10-5). Metabolomics profiles might be useful for assessment of dietary intake in cohort studies, particularly those related to dietary patterns.

POSTER 105

Exposome-Explorer, a new database on biomarkers of exposure to dietary and other environmental factors for epidemiologists and clinicians

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Biomarkers constitute an objective approach to assess exposure to lifestyle and environmental factors and evaluate their associations with health and diseases in epidemiological studies. Most often researchers have measured single or small sets of biomarkers. However the etiology of chronic diseases often results from exposures to quite diverse risk factors, which constitute altogether the exposome. We developed Exposome-Explorer, a new database collating all available information on biomarkers of exposure and their state of validation. Highly detailed information on biomarkers of dietary exposure measured in population studies was systematically collected from peer-reviewed publications. It includes a description of the populations and subjects in each study, samples analyzed, methods used for biomarker analyses, concentrations in biospecimens, correlations with external exposure measurements and confounders. The database and the web interface to insert, validate and retrieve data were developed in MySQL and Ruby on Rails. The content of 196 publications was analysed and 2,985 concentration values in blood, urine and other biospecimens for 131 dietary biomarkers (53 fatty acids, 7 amino acids, 16 vitamins, 27 polyphenols, 13 carotenoids, 7 alkylresorcinols and 1 carboxylic acid) were extracted. These biomarkers have been used to assess exposures to food nutrients/bioactives or as surrogate markers for food intake (fruits, vegetables, cereals, dairy foods, meats, fish, beverages, nuts). Information on the state of validation of the biomarkers was also collected. Exposome-Explorer notably contains over 8,000 correlations values between biomarker levels and nutrient or food intake in various populations. Methods for biomarker measurement and dietary assessment are also documented. Links to chemical databases (PubChem, CAS) and PubMed are provided. Additional information on pharmacokinetics, biomarker stability over time and dose-response relationships is also being compiled. Exposome-Explorer will be freely accessible online and various queries will be made possible to retrieve for example all dietary biomarkers that can be measured in urine by LC-MS, or to compare correlations with intake for all biomarkers measured in different biospecimens. The database will be extended to also include pollutants and contaminants. Exposome-Explorer should be very useful to define panels of biomarkers that capture at best a significant fraction of the exposome in epidemiological studies and to evaluate its association with disease risk in exposome-wide association studies. Exposome-Explorer is the first existing database on biomarkers of exposure to environmental factors.

POSTER 106

Direct infusion MS-based lipid profiling reveals the pharmacological effects of compound K-reinforced ginsenosides in high-fat diet induced obese mice

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Obesity and hyperlipidemia induced by high-fat diets (HFD) are the most important risk factors for development of diabetes mellitus, and fatty liver. Therefore, treatment of obesity and hyperlipidemia must be addressed as part of a healthy lifestyle. Efforts to prevent obesity and hyperlipidemia with herbal medicines such as ginseng are underway. Various in vivo studies have suggested that ginseng may exert anti-obesity and hypolipidemic effects. However, the precise biochemical mechanisms of action of ginseng in these diseases remain poorly understood. In the present study, we used mass spectrometry (MS)-based serum lipid profiling and conventional serum chemical analysis to explore the biochemical abnormalities in obese mice fed HFD, and determined the pharmacological effects of compound K-reinforced ginsenosides (CK) in such mice. The mice were divided into three groups according to the diet type; normal diet (ND), high-fat diet (HFD), and high-fat diet plus CK at 400 mg/kg (HFD+CK). Serum lipids were extracted using the methyl-tert-butyl ether. The extracted lipids were analyzed by the direct infusion nanoelectrospray-ion trap mass spectrometry. Nominal ion mass spectra data from the ion trap mass spectrometer were processed by the Genedata Expressionist MSX module. In addition, serum lipids were identified by comparison with the MS/MS fragmentation patterns of commercially available standards or using the LIPID MAPS Lipidomics Gateway, the Human Metabolome Database, and/or our

in-house Lipid library (LipidBlast). Multivariate analysis was performed to explore the extent of differences among the three groups. The obese and lean groups were clearly discriminated upon PCA and PLS-DA score plot and the major metabolites contributing to such discrimination were triglycerides (TGs), cholesteryl esters (CEs), phosphatidylcholines (PCs) and lysophosphatidylcholines (LPCs). We also found that TGs with high total carbon number (> 50) and low total carbon number (< 50) were negatively and positively associated with HFD induced obesity and hyperlipidemia in mice, respectively. It has been reported that diabetes- and obesity-induced changes in hepatic lipid compositions correlated with changes in the levels of fatty acid elongase and desaturase. HFD significantly suppressed expression of Elovl-5 (a key enzyme of polyunsaturated fatty acid synthesis) by inhibiting the expression of nuclear sterol-regulatory element binding protein-1 (SREBPOSTER 1). Based on these findings, we suggest that the carbon number and double bond values of TGs may serve as very useful markers of metabolic diseases. When the CK fraction was fed to obese mice that consumed a HFD, the levels of certain lipids including LPCs and CEs became similar to those of mice fed a normal diet. The biosynthesis of CEs is regulated by stearoyl-CoA desaturase (SCD1). Compound K attenuated expression of the gene encoding SREBPOSTER 1c. Therefore, the fall in CEs levels in the HFD+CK group (compared to the HFD group) may be attributable to down regulation of SREBPOSTER 1c by compound K. The levels of LPCs increased on ingestion of an HFD but were significantly reduced in mouse serum if the diet contained compound K. LPCs, major lipid constituents of oxidized low-density lipoprotein (LDL), are generated via hydrolysis of the lipid components of oxidized LDL. Earlier studies found that LDL oxidation was inhibited by ginsenosides. Therefore, our work highlights the fact that the reduced LPC levels in the HFD+CK group may be attributable to an effect of compound K, a metabolite of ginsenoside Rb1. These lipid biomarkers (LPCs and CEs) are responsible for pharmacological effect of compound K in high-fat diet induced obese mice.

POSTER 107

Metabolomics Approach to Determine the Roles of Soybean Oil and HNF4α in Obesity

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The incidence of obesity in the U.S. has increased from 15% to 35% in the last 40 years and is expected to rise to 50% by 2030. One factor that is not well studied but correlates with the obesity epidemic in the U.S. is a 1000-fold increase in consumption of soybean oil (SO), which contains ~55% linoleic acid (LA), a C18:2 PUFA: thus the daily energy intake from LA in the U.S. has risen from 2% to >7% between 1909-1999. LA is an essential fatty acid and we recently identified it as an endogenous ligand for HNF4α, a highly conserved nuclear receptor that is linked to diabetes and a master regulator of liver-specific gene expression. To investigate the role of SO and HNF4α in the obesity epidemic, we treated C57/BL6 mice and HNF4α exon-swap mice with specialized isocaloric diets that are moderately high in saturated fats (40%kcal) with or without supplementation of SO to achieve 2% kcal LA (HFD) or 10% kcal LA (LA-HFD). HNF4α exon-swap mice are genetically engineered to express just the P2-form of HNF4α so that in tissues, such as liver, where the P1 promoter is active, HNF4α7/8 is expressed instead of HNF4α1/2. Using an integrated multi-omics approach, liver samples from these mice were analyzed at the NIH West Coast Metabolomics Center, UC Davis to determine alterations in primary metabolites, complex lipids, oxylipins and protein expression induced by the different diets. When C57/BL6 (WT) male mice are weaned onto LA-HFD, they become more obese, more diabetic and glucose intolerant, and more insulin resistant (IR) than WT mice on HFD, despite the fact the diets are isocaloric and food consumption is roughly equivalent. The livers of the LA-HFD mice also have large lipid droplets and balloon injury, reminiscent of human NAFLD, while the moderate HFD livers have less lipid accumulation and no balloon injury. These results suggest that SO may be more obesogenic than coconut oil. On the other hand, the exon swap mice on LA-HFD did not develop obesity, diabetes, IR or fatty liver as did the WT mice on LA-

HFD indicating that the exon-swap mice are resistant to the metabolic effects of SO and that HNF4α may play a major role in the pathogenesis of obesity. We will present a meta-analysis of the metabolomics data obtained from the livers of WT HFD, WT LA-HFD and exon-swap LA-HFD mice in order to elucidate the molecular mechanism by which SO drives various aspects of the metabolic phenotype as well as the role of HNF4α in SO-induced metabolic syndrome. To our knowledge this is the first in-depth analysis of the role of soybean oil and HNF4α in metabolic syndrome.

POSTER 108

Investigation of metabolic changes in a mouse model of pneumonia caused by Streptococcus pneumoniae using an high-resolution 15T FT-ICR MS

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We investigated metabolite changes in a mouse model of bacterial pneumonia to understand host metabolic responses upon bacterial infections. Here we focused on pneumococcal pneumonia that is caused by Streptococcus pneumoniae, a facultative anaerobic gram-positive bacterium. The pneumonia can be mild or serious, even leading to respiratory failure or death; therefore, early diagnosis of pneumonia using pneumonia-specific biomarkers is crucial for effective treatment of the infection with antibiotics, resulting significant decrease of the danger of acute respiratory distress. In this study, we used a mouse pneumonia model infected by Streptococcus pneumoniae, then introduced metabolomics approaches to investigate metabolite changes in the murine model upon microbial infection. Methanol extracts from serum or lung of pneumonia mice were analyzed using a 15 Tesla Fourier transform ion cyclotron resonance (15 T FT-ICR) mass spectrometry to obtain ultrahigh-accuracy profiles in both positive and negative ion modes. Normal mice samples of serum or lung were used for control experiments. The multivariate data analyses such as principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were employed to differentiate the key metabolites between normal and pneumonia-induced mice. Using the FT-ICR MS profiles with a resolution of 400,000 at m/z 400, hundreds of metabolites were predicted from biological samples of normal and pneumonia-induced mice. Among those, more than 100 metabolites could be identified and quantified to investigate their fold changes upon pneumococcal pneumonia. The results of PCA and PLS-DA also suggested key metabolite factors differentiating normal and pneumonia mice. Key metabolites observed in the murine pneumococcal pneumonia model would lead to the development of potential diagnosis for bacterial pneumonia.

POSTER 109

An integrative study of regulatory lipid mediators' metabolic pathway in a murine asthmatic model

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Regulatory lipid mediators are a class of signaling lipids, which regulate various physiological and pathological processes including inflammation, angiogenesis, immune response etc. Over 75% of the medicine in mass is targeting in metabolic pathways related to these regulatory lipid mediators. The current study describes an integrative study in a murine asthmatic model. In this study, metabolic profile responses in different organs and bio-fluids as well as the mRNA level and protein level changes of the enzymes in these pathways were compared among control, disease and after different pathway blocked

with inhibitors. BALB/c mice were sensitized to OVA over 4 weeks and exposed to 1% OVA aerosol over 2 weeks. The soluble epoxide hydrolase inhibitor (1 mg/kg) and MK886 (a 5-lipoxygenase activation protein inhibitor) (10 mg/kg) were administrated by subcutaneous injection for two weeks starting two days before ovalbumin exposure. A selected panel of cytokines and chemokines in the lung lavage was measured by a Multiplex cytokines kit. An established liquid chromatography tandem mass spectrometry method was used to measure 87 regulatory lipids mediators (including prostaglandins and leukotrienes) for plasma, lung tissue homogenates, and lung lavage. The regulatory lipid mediator profiles were distinct in circulation (plasma), adjacent site (lung tissue) and activation fluids (lung lavarge). The blockage of enzymes in each of the pathway caused the complex response in related pathways. A mass shunt was noticed in general but also the pro-inflammatory mediators were decreased along with the pro-inflammatory cytokines and chemokines reduction. Along with the metabolic profile changes, the mRNA level of the relevant enzymes also showed down regulated when inhibitors were given. In general, these inhibitors reduced the pro-inflammatory lipid mediators in vivo, leading to less inflammation and pro-inflammation enzymes expression. As a result, less pro-inflammatory lipid mediators were generated. All above results fit well with the phenotypes changes with pharmacological inhibition of sEH and FLAP. Both sEHI and FLAP inhibitor reduced fractional exhaled NO (FeNO) levels, total live cells number in lung lavage and eosinophil percentage in lung lavage of OVA-exposed mice. The Th2 cytokines (IL-4, IL-5) and chemokines (Eotaxin and RANTES) were dramatically reduced after administration of sEHI. Furthermore, combination of lower dose of sEHI and FLAPi reduced these inflammatory markers more. Moreover, the resistance and dynamic lung compliance were also improved by pharmacological inhibition of sEH and FLAP. An integrative study including metabolic profiles in different location, mRNA of relevant enzymes were done in a murine asthmatic model.

POSTER 110

HIGH-RESOLUTION METABOLOMICS OF AIRWAY FLUID FROM ADULT CYSTIC FIBROSIS SUBJECTS: INSIGHTS INTO A METASTABLE, PATHOLOGICAL HUMAN AIRWAY ECOSYSTEM

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In cystic fibrosis (CF), the commonest fatal recessive disease in Caucasians, neutrophilic inflammation and microbial colonization lead to airway destruction. CF airway disease is metastable, interspersed with acute pulmonary exacerbation (APE) reactions to environmental stressors, e.g., cold, de novo infection, or planktonic pioneers from established bacterial biofilms. The composition of airway fluid is a crucial determinant of CF pathophysiology. In particular, we and others have shown that metabolites accumulating in CF airway fluid drive adaptations in both microbes (auxotrophy for selective amino acids) and neutrophils (anabolic reprogramming, featuring accelerated glucose and amino acid metabolism and concomitantly increased lifespan). To date, no study has provided a global picture of CF airway fluid metabolomics, a gap that we intend to fill here. This pilot study included 14 CF patients hospitalized for an APE, with follow-up assessment 3 months later (metastable state). Corresponding purified sputum supernatant samples were analyzed in triplicate using a high-resolution Thermo Orbitrap Velos mass spectrometer in positive and negative ion modes after acetonitrile extraction containing an internal standard mixture and C18 column separation. We used apLCMS and xMSanalyzer tools to provide an feature table of detected metabolites, denoted by accurate mass (m/z) and associated retention time and intensity values within individual subjects at each timepoint. Statistical analyses included false discovery rate analysis using LIMMA, and partial least square discriminant analysis (PLSDA). Metlin and KEGG metabolite databases were searched for differentiating metabolites; pathway enrichment interrogation was performed using the Mummichog program. Several hundred metabolites, including amino acids, inflammatory lipids and nucleotides, were detected in CF sputum in both negative and positive ion modes, with amino acids and dipeptides predominating. PLSDA showed a clear separation of matched APE and post-recovery metastable samples. Pathway analysis using Mummichog identified metabolites from the

following pathways, as being significantly different between acute and metastable samples: (i) in negative ion mode, glycolysis and pentose phosphate pathway (PPP), amino acid, purine and pyrimidine, and glycerophospholipid metabolism; (ii) in positive ion mode, the same core pathway differences were identified; however, with a preponderance of amino acid metabolic pathways. Prior targeted biochemical and metabolic profiling efforts in CF airway fluid have identified these same pathways as being important in CF pathogenesis, notably linked to neutrophilic inflammation. Remarkably, the dipeptide carnosine (beta-alanyl-L-histidine) was found in both negative and positive ion modes to be highly upregulated in APE compared to matched metastable samples. Carnosine has ben previously linked to activation and increased lifespan of neutrophils, but was not previously identified in the airway milieu. This finding corroborates our previous identification of a pro-survival bias in neutrophils resident in CF airways. In addition, the amino acid glutamine was significantly upregulated in airway fluid at APE vs. metastable state; this finding is consistent with our prior description of glutamine transporter ASCT2 as key to neutrophil reprogramming in CF airways. Parallel direct profiling of amino acids using a targeted GC/MS platform, as well as selected verification studies using tandem MS/MS analysis (e.g., for carnosine) are in progress. In addition, metabolomics analysis of a second, independent cohort of clinically similar patients over time (matched APE and metastable state visits) will further validate these findings. Supported by the Emory HERCULES Exposome Center grant NIEHS: P30 ES019776. This study identifies candidate pathways associated with CF airway ecosystem destabilization during APEs, opening avenues for further research.

POSTER 111

High-resolution metabolomic profiling can distinguish multidrug resistant pulmonary tuberculosis in human plasma

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Multidrug resistant (MDR-TB), extensively drug-resistant (XDR-TB) and now totally drug-resistant tuberculosis are threatening TB care infrastructure. In 2013, 480,000 people developed MDR-TB and XDR-TB was reported in 100 countries. The World Health Organization has recommended the same drug regimen since 1965 and recent drug targets have been unsuccessful. Metabolomics methods have been successful in distinguishing TB subjects from healthy controls. It is unknown whether plasma metabolomics can distinguishing MDR-TB from drug susceptible TB (DS-TB). We aimed to use plasma high-resolution LC-MS metabolomics to differentiate these newly diagnosed pulmonary TB subjects enrolled in a double-blind randomized controlled trial of high dose vitamin D3 versus placebo in Tbilisi, Georgia. Plasma from 23 patients with MDR-TB and 45 age- and gender-matched patients with DS-TB were obtained within 7 days of initial TB disease diagnosis (baseline), and 4- and 8-weeks. Plasma was analyzed using high-resolution LC-MS. To conduct the metabolome-wide association study (MWAS) for significant features distinguishing between MDR-TB and DS-TB patients at each time point, we fit individual regression models for each feature, with drug susceptibility status as the independent variable and the feature intensity as the dependent variable, adjusting for diabetes status, income, BMI, sex, age, treatment group (vitamin D vs placebo), serum 25-hydroxyvitamin D concentration and batch. Statistically significant (Poster value < 0.05) features were further input into Mummichog, a software tool designed for

high-throughput metabolomics, for pathway analysis. In both TB groups, the average age was 34±9 yrs old, and ≈ 50% were male. In this cross-sectional analysis, we found 246, 216 and 229 significant metabolites that differed between MDR-TB and DS-TB patients at baseline, week 4 and week 8 of conventional anti-TB treatment, respectively, under raw Poster value < 0.05. At baseline (prior to Vit D or placebo), pathway analysis revealed significant differences between subjects with MDR-TB vs DS-TB in glutamate metabolism [p = 0.02], retinol metabolism [p = 0.02], cytochrome P450 metabolism [p = 0.03], pyrimidine metabolism [p = 0.05], tryptophan metabolism [p = 0.05], urea cycle metabolism [p = 0.05], and leukotriene metabolism [p = 0.02]. Several potential m/z matches were upregulated in MDR-TB, including 1-pyrroline-5-carboxylate [m/z 114.054 RT 251], which has been linked to Mycobacterium tuberculosis as a respiration intermediary metabolite. Retinol metabolism metabolites were upregulated in MDR-TB; this is of interest as vitamin A has been linked to anti-mycobacterial activity. Urea cycle related metabolites were upregulated in MDR-TB, which could reflect the catabolic effects of more severe infection. The leukotriene LTB4 was down-regulated in MDR-TB, which may be linked to suppressed immune function in MDR-TB relative to DS-TB. High-resolution plasma metabolomics distinguished MDR-TB subjects from their age- and gender-matched DS-TB controls. Metabolites of interest need to be verified.

POSTER 112

exploratory analysis to detect effects of smoking and smoking cessation on community-dwelling Japanese serum metabolite profile; Tsuruoka Metabolomic Cohort Study

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In cohort study, many important factors are measured as categorical variable. Our cohort collect life style data relating sleeping status, diet, exercise and smoking status (number of cigarettes smoked,smoking/cessation period, etc.). When we conduct statistical analysis, we need to assumption that population within same level of categorical variable is homogeneous. We focused on smoking status (non-smoker / ex-smoker / current-smoker). First of all, we detect smoking related metabolome profile by comparing metabolite between non-smoker and current-smoker in our study population. Nest, we explore what kind of life style factors to effect smoking related metabolome profile in ex-smoker population, whose metabolome profile distributed between non-smoker type and current-smoker type. Recruitment period of our study was April 2012-Mar 2015. Our subjects will be 10,000 residents and worker in Tsuruoka city, Japan. We collected plasma and urinary metabolites from 12-h fasting subjects. Total 115 metabolites (Cation 63, Anion 52) was measured from 2,126 subjects (Male 1,017, Female 1,109). Life style status relating sleeping status, diet, exercise and smoking status was measured via questionnaires. To detect smoking related metabolome profile, we compare 115 metabolites between non-smoker and current-smoker (P<.0001), and conduct principal component analysis to explore the structure of data distribution. Next, we compute principal component score with ex-smoker subjects, and discriminate they are non-smoker type or current-smoker type profile, and detect which life style effects profile type using linear regression model. In our study, 197 (19.4%) male and 1,004 (90.5%) female are non-smoker, 541 (53.2%) male and 72 (6.5%) female are ex-smoker and 279 (27.4%) male and 33 (3.0%) female are current-smoker subjects with self reported questionnaire. We detect 7 metabolite (Pipecolate, Trigonelline, Glu, 2-Hydroxybutyrate, 3-Hydroxybutyrate, Guanidinosuccinate, CSSG) in male and 4 metabolite (Ala, Trigonelline, 2-Hydroxybutyrate, 3-Hydroxybutyrate) in female to explain difference between non-smoker and current-smoker. This metabolome profile have ability to discriminate smoking status 0.64 AUC (area under the ROC curve). We classify ex-smoker metabolite into non-smoker type or current-smoker type, and compare life style between these 2 type subjects, who is ex-smoker but have non-smoker type metabolite profile or current-smoker type metabolite profile. We detect cessation period (P=0.018), age (P=0.006), secondhand smoke (P=0.048), sleep time (P=0.049), eating speed (P=0.039), more than 1-hour exercise per day (P=0.046), more than 10kg weight gain from 20yrs (P=0.006). We reveal what kind of life style to accelerate and improve metabolome profile from current-smokertype to non-smoker type.

POSTER 113

Interrelationship of metabolomics pathway markers of Asthma in a mouse model

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At some point in their lives, at least 12% of the population within the United States will suffer from asthma. For certain racial groups, the number can be higher than 20%. This chronic disease is multifactorial in nature making it difficult to uncover its underlying mechanisms. Due to close proximity to the lung, bronchoalveolar lavage (BAL) fluid can be studied to identify markers of disease. However, the collection procedure is invasive, and not always feasible in humans. To understand what is occurring in asthmatics, BAL fluid from a mouse model of experimental asthma, and matching plasma were analyzed using untargeted metabolomics to identify small molecules and pathways involved in disease. Pathogen-free female C57BL/6 mice were sensitized to and challenged with ovalbumin resulting in allergic asthma-like symptoms. Airway hyperresponsiveness measurements and differential cell counts were performed to confirm asthma-like symptoms in the mice. Bronchoalveolar lavage fluid and plasma were collected and prepared using liquid-liquid extraction to separate aqueous and hydrophobic metabolites. The aqueous fraction was separated using a HILIC column and analyzed on an ESI-QTOF in positive mode, the lipid fraction was separated on a C-18 column and analyzed on an ESI-TOF in both positive and negative modes. Spectral data was extracted using Profinder software (Agilent), and statistical analysis was performed using Mass Profiler Professional (Agilent). Metabolites which passed significance levels ($p<0.05$) were imported into KEGG for pathway analysis. Invasive airway hyperresponsiveness measurements were obtained for all the mice. 48 hours after the final challenge, the mice that were sensitized to and challenged with ovalbumin (OVA/OVA) showed a significant increase ($p\leq0.05$) in lung resistance relative to mice that were sensitized but not challenged (OVA/PBS) and the mice that were not sensitized but were challenged (PBS/OVA). A significant increase ($p\leq0.05$) in eosinophils, a marker of allergic asthma, was observed in the OVA/OVA mice relative to the OVA/PBS and the PBS/OVA mice. Liquid-chromatography mass spectrometry-based metabolomics was used to determine dysregulated metabolites among groups. Statistical analysis was performed on BAL and plasma to compare the OVA/OVA, OVA/PBS and PBS/OVA experimental groups at 6, 24, and 48 hours following the final challenge. Analysis revealed that the greatest number of dysregulated metabolites was at the 6 hour time point in the BAL compared to the 48 hour time point in the plasma, suggesting a more direct measurement at the lung in BAL fluid compared to downstream effects in plasma. Statistically significant metabolites ($p\leq0.05$) in both biofluids were mapped to four pathways. These include sphingolipid metabolism, glycerophospholipid metabolism, arginine and proline metabolism, and Neurotrophin signing pathway. Interestingly, all four pathways were related by a mere four metabolites - nitric oxide, serine, glycine and creatinine of which nitric oxide has been repeatedly shown to be a marker of asthma. An interrelationship between multiple dysregulated metabolomic pathways was observed using a mouse model of allergic asthma.

POSTER 114

Dysregulated Pathways in Human and a Mouse Model of Chronic Obstructive Pulmonary Disease

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Chronic obstructive pulmonary disease (COPD) is currently the third leading cause of death in the United States. COPD is a chronic lung disease typically affecting cigarette smokers; no cure is available and treatment is limited. In preliminary studies, sphingolipids were determined to be significantly dysregulated

in a small subset of human COPD patients; these molecules were also dysregulated in a cigarette smoking (CS) mouse model of emphysemous COPD. To fully explore the mechanisms of COPD, plasma was analyzed from mice in a COPD mouse model and compared to the plasma from human COPD subjects. Our aim was to identify overlapping dysregulated pathways in both species with the long term goal of determining potential treatments for disease. Plasma, from subjects selected from the COPDGene cohort, was collected from current and former smokers (n=131) aged 45–80 years with a history of smoking at least 10 pack-years. Subjects with a ratio of forced expiratory volume in 1 second (FEV1) to forced vital capacity (FVC) <0.70 were designated as COPD and divided into GOLD stages I-IV. Plasma was also collected from C57BL/6 mice exposed to ambient air or cigarette smoke for 9 months (n=10 mice/group). Protein precipitation and organic liquid-liquid extraction was performed on plasma to obtain aqueous and lipid fractions; these then underwent LC/MS-based metabolomics. Metabolites were annotated using public and in-house databases. Quantitative and statistical software was utilized to identify differences in metabolites among groups. Over 4500 metabolites were detected in both the mouse and human plasma samples. Statistical analysis comparing the nine month air control to the nine month smoking mice groups resulted in 147 statistically significant metabolites ($p \leq 0.05$) in the mouse plasma. Pathway analysis revealed ten perturbed pathways which met statistical significance ($p \leq 0.05$). These included glycerophospholipid metabolism (p=2.04e-7), glycosylphosphatidylinositol-anchor biosynthesis (p=1.07e-4), regulation of autophagy (p=3.55e-4), phosphatidylinositol signaling system (p=5.33e-4), inositol phosphate metabolism (p= 0.0232), glycerolipid metabolism (p=8.86e-4), and purine metabolism (p=0.0176). For human datasets, differentially regulated metabolites were identified using linear regression modeling for FEV1 percent predicted (FEV1PP) in R and adjusted for covariates including age, gender, and BMI. The 455 statistically significant metabolites ($p \leq 0.05$) that were identified were mapped to fourteen pathways with $p \leq 0.05$. These included glycerophospholipid metabolism (p=1.45e-11), sphingolipid metabolism (p=3.20e-11), phosphatidylinositol signaling system (p=1.14e-4), glycerolipid metabolism (p=2.25e-4), glycosylphosphatidylinositol-anchor biosynthesis (p=2.76e-4), pathways in cancer (p=8.67e-4), regulation of autophagy (p=9.09e-4), inositol phosphate metabolism (p=0.05), and adipocytokine signaling pathway (p=0.00188). From these results, six pathways were common to and significant ($p \leq 0.05$) in both the mouse and human data; this validates both the strength of the mouse model and the power of untargeted global profiling. This approach can therefore be used toward furthering the understanding of the mechanisms of COPD. Metabolomics profiling revealed significant overlap in perturbed pathways in both human COPD patients and a mouse model of emphysemous COPD.

POSTER 115

A preliminary mass spectrometry based metabolomics study highlights the role of inflammatory lipid mediators in adult asthma.

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Asthma is a chronic pulmonary disease that affects an estimated 235 million people worldwide. Chronic airway inflammation, the hallmark feature of asthma, leads to airway constriction, which reduces airflow, and smooth muscle contraction. Common symptoms of asthma include recurrent attacks of breathlessness, wheezing, chest tightness, and coughing. The mortality rate for asthma is low; however, the morbidity is quite high and is associated with substantial individual burden due to secondary effects such as restricted activity levels and sleeplessness. The underlying disease mechanisms in asthma are heterogeneous and not completely understood; this represents a major barrier in treatment. HYPOTHESIS: A semi-targeted mass spectrometry based metabolomics study will provide insights into the pathogenic mechanisms of asthma, thereby identifying novel therapeutic targets. Serum samples were collected from healthy controls (N=22), mild (N=12), moderate (N=20), and severe (N=24) asthma patients, classified a priori by clinical measures. Proteins were removed by precipitation and samples were analyzed using high-resolution LC-MS employing a combination of reversed-phase and HILIC

chromatography coupled to a Q-Exactive Orbitrap mass spectrometer in positive and negative ionization modes. Sequence order was randomized and pooled quality control (QC) samples were injected after every 6th sample. Metabolites were identified based on matching accurate mass and retention times to an authentic chemical reference database of 700 compounds. Prior to data analysis, signal correction and data cleaning were performed; metabolites with relative standard deviation (RSD) <25% in the QC samples were included in data analyses. A total of 108 metabolites were included in the final statistical analysis, comprising lipids (free fatty acids, eicosanoids, ceramides, sphingolipids), energy metabolites, amino acids and biogenic amines, excretion metabolites, and exogenous compounds. Univariate analysis, followed by false discovery rate correction using the Storey method, revealed that linoleic acid, palmitoleic acid, 13-hydroxyoctadecadienoic acid (13-HODE), and 12(13)-epoxy-octadecadienoic acid (12(13)-EpODE) were significantly increased (FDR-adjusted $p<0.05$) in mild and severe asthma phenotypes versus healthy controls. Metabolites that were significantly different (FDR-adjusted $p<0.05$) between only two groups included: ceramide (18:0) (increased in mild asthma versus healthy controls), oleic acid (increased in severe versus moderate asthma), and pipecolate (decreased in severe asthma versus health controls). Principal Component – Canonical Variate Analysis (PC-CVA) was used to assess correlated discrimination between the four clinical groups. The first canonical variate (CV1) revealed that: (1) moderate asthma was metabolically very similar to healthy controls and (2) there was a mean difference between healthy controls/moderate asthma and mild/severe asthma phenotypes. Bootstrap resampling/remodelling revealed that linoleic acid, palmitoleic acid, 13-HODE, and 12(13)-EpODE significantly contributed to separation along CV1. Linoleic acid is a pro-inflammatory omega-6 polyunsaturated fatty acid (PUFA) common in western diets. 13-HODE, an oxidation product of linoleic acid, is an endogenous agonist of peroxisome proliferator-activated receptor-gamma (PPAR-gamma); existing reports are not conclusive, but this activation plays an important role in mediating T-cell activation and differentiation, likely toward the TH2 phenotype, which is known to be involved in asthma pathogenesis. Linoleic acid and 13-HODE are associated with mild and severe asthma, possibly through the development of a TH2 phenotype.

POSTER 116

Deregulated fatty acid profile in pulmonary tuberculosis patients.

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Understanding molecular dynamics of biofluids of patients provide information of deregulated molecules that may be useful in developing adjunct therapy for better disease management. Serum fatty acid compositions have provided useful insight into many metabolic disorders like diabetes, obesity etc. In pulmonary tuberculosis (TB), although primary organ affected in TB is lungs we do expect alterations in different fatty acid and other lipid profiles in the circulatory fluids of these patients. In this study, we attempted to explore if any difference between fatty acid profile of tuberculosis (TB) patient and asymptomatic healthy individuals exist or not. Serum samples (50 ml) from age and gender matched TB patients (n=20) and healthy subjects (n=20) were processed to methyl esterify bound (EFA) and free fatty acid (NEFA) using base and acid catalyzed reaction respectively. Methyl esterified samples were analyzed using a gas chromaptography-time of flight mass spectrometry (GC-ToF-MS) system and unbranched fatty acids are validated using commercial standard mix run. Peak aligned files were used to explore patterns between study groups. Samples from subjects were collected after receiving written informed consent to participate in this study and study protocols were approved by Institute Review Board of both study sites. Using the adopted method we successfully identified and validated ~ 20 fatty acids both in bound and free form from all study samples. Multivariate analysis using partial least square discriminate analysis showed certain pattern do exist between serum fatty acid profiles of TB patients and healthy subjects. Further analysis showed these differences are attributed by both individual fatty acid

abundances as well as the EFA/NEFA ratio. Comparing the desaturase index of these groups also provide additional details on role of fatty acid in inflammation in TB. Although in this current study sample size is low, our ongoing effort is to further validate these findings in large scale population study. Serum fatty acid profile, an unexplored metabolic component of TB patients, provides useful information to understand disease biology.

POSTER 118

Metabolomics of Neonatal Pulmonary Hypertension

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Bronchopulmonary dysplasia (BPD) is a serious and common chronic lung disease of infancy. Pulmonary hypertension (PH) is a common complication of BPD that contributes to worse clinical outcomes and significantly increases the morbidity and mortality of preterm birth. PH is often not diagnosed in premature infants until symptoms emerge after many weeks of life, when the disease is advanced and associated with severe cardiac dysfunction. However early screening methods have not proven to be reliable predictors of PH and no biomarkers currently exist. A recent clinical study suggests that preterm infants with BPD and low birth weight are at increased risk of developing PH. The aim of this study is to identify novel biomarkers associated with BPD and PH. We combined an established rat model of BPD and PH, achieved by exposing newborn pups to 75% oxygen, with a model of postnatal growth restriction, achieved by increasing litter sizes from 10 to 17 pups. We hypothesized that growth restriction would exacerbate oxygen-induced PH. After 14d pups were sacrificed, heart and lungs snap frozen in liquid nitrogen and blood plasma collected and stored. Hearts and lungs were analyzed for evidence of PH. Lung and plasma samples were analyzed by the West Coast Metabolomics Center to detect primary metabolites by GC-TOF MS, and to detect complex lipids and oxylipins by UPLC-MS/MS. Archived cord blood from preterm infants with and without PH was also submitted to WCMC for analysis. Growth restriction or oxygen exposure independently induced PH as evidenced by right ventricular hypertrophy (RVH) and increased pulmonary artery wall thickness in 14d-old rats. Growth restriction and oxygen combined increased RVH and medial wall thickness further relative to either stress alone. Oxidant stress induces pulmonary hypertension via multiple mechanisms resulting from elevated levels of reactive oxygen species (ROS). Our preliminary data indicate that total ROS levels were elevated in the lungs of 14d-old rats exposed to growth restriction or oxygen, and ROS levels were elevated further in the lungs of rats exposed to both stresses. Metabolomics analysis of oxylipins in rat lung and blood samples indicate that growth restriction and oxygen increased the levels of distinct biomarkers of oxidant stress. For example, oxidation products of arachidonic acid include hydroxyeicosatetraenoic acids (HETEs) and dihydroxyeicosatrienoic acids (DiHETrEs); 5-HETE was increased in rats exposed to oxygen, 9-HETE was increased in rats exposed to growth restriction, while 5,6-, 8,9-, and 11,12-DiHETrEs were increased by oxygen or growth restriction and elevated further when both stresses were combined. Analysis of primary metabolites indicated that growth restriction and oxygen decreased lung and blood levels of citrulline, a precursor of arginine required for NO-mediated pulmonary vasodilation. Analysis of complex lipids indicated that in general, growth restriction decreased lysophosphatidylcholines while oxygen increased diacylglycerides and fatty acids in lung and blood. Analysis of cord blood from preterm infants with PH is currently underway. Our preliminary data indicate that growth restriction and oxygen may induce PH in neonatal rats via distinct and common signaling pathways. The generation of biochemical network maps and comparisons with data from patient cord blood samples may improve our knowledge of the underlying mechanisms that induce PH, and may identify novel biomarkers that predict the risk of developing PH before the disease is advanced. Our data provide for the first time a metabolomics profile of infants with PH and a novel model of PH.

POSTER 119

Metabolic changes in human fibroblasts isolated from Idiopathic pulmonary fibrosis patients

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Idiopathic pulmonary fibrosis (IPF) is characterized with excessive extracellular matrix deposition in the lung and changes attributed to associated hypoxia. There is mounting evidence to show that cells exposed to hypoxia alter their metabolism to survive and this response to hypoxia has an imperative function in the pathogenesis of many diseases, as exemplified by ischemic disease following stroke or myocardial infarction and in tumorigenesis as well. Although studies with regulatory factors such as hypoxia inducible factor-1 (HIF-1), Poly [ADPOSTER ribose] polymerase 1 and Sirtuin 1 etc. implied the potential for metabolic alterations in lung cells contributing to the progression of pulmonary fibrosis, direct metabolomic evidence is lacking. In this study, the glycolytic pathway and tricarboxylic acid (TCA) cycle metabolites in fibroblasts isolated from IPF patients or control subjects were analyzed by LC/MS, GC/MS and confirmed by ELISA assays. The results showed that most components of the glycolytic pathway and TCA cycle, including fructose 6-phosphate, glucose 6-phosphate, pyruvate, α-ketoglutarate (α-KG), fumarate, malate and oxaloacetate, as well as hexose monophosphate shunt metabolites, ribose-5-phosphate and sedoheptulose 7-phosphate were significantly increased in fibroblasts isolated from IPF patients relative to cells from control subjects. Cofactor nucleotides including ATP and NADH were significantly decreased, while FAD, ADP, AMP, NADP, NADPH were increased significantly in IPF lung fibroblasts. Further studies with a HIF-1 prolyl hydroxylase (PHD) competitive inhibitor, dimethyloxaloylglycine (DMOG) or cofactor, α-KG in vitro revealed the importance of this TCA metabolite in regulation of myofibroblast differentiation by modulation of HIF-1 stability/degradation. Thus, metabolic regulation of myofibroblast differentiation during the pathogenesis of idiopathic pulmonary fibrosis possibly mediated by HIF-1 is suggested.

POSTER 120

Metabolic Alterations in Patients with Alzheimer's Disease.

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Alzheimer's Disease (AD) currently affects more than 5 million Americans, with numbers expected to grow. The pathophysiological changes in AD patients begin decades before the onset of dementia, highlighting the urgent need for the development of early diagnostic methods. Using non-targeted and targeted metabolomic approaches, we reported presence of signatures of early energetic stress and mitochondrial dysfunction in the brain tissue of multiple transgenic animal models of AD, and plasma and cerebrospinal fluid of patients with AD. To determine the exact mechanism of alterations, we utilized stable isotope tracers ([U-13C]-glucose and [U-13C,15N]-glutamine) and mass spectrometry to measured the TCA flux in primary fibroblasts from male and female patients of AD. Using stable isotope tracers and we examined alterations in glucose metabolism in primary human fibroblasts from patients with sporadic AD and age- and sex-matched control individuals. Using [U-13C]-glucose as a tracer, we determined the extent of both glucose consumption and lactate production, and metabolism through the TCA cycle. Using [U-13C,15N]-glutamine as a tracer, we examined glutamine import and metabolism through the TCA cycle in AD patient fibroblasts relative to control cells. These data provide the insights in whether glutamine is utilized for an alternative glucose- independent TCA cycle. Data will be compared to our previous results obtained in the CSF and plasma from MCI and AD patients. Based on the changes identified in the TCA cycle in AD compared to control fibroblasts using stable isotope-labeled glucose and glutamine tracers, we expect to identify the relationship between glucose-dependent and -independent TCA cycle activity. We will discuss to what extent different patients with sporadic AD share similar alterations in the TCA cycle, and to whether these changes are consistent with changes in metabolites identified in the CSF and plasma in our previous work. We will also discuss to what extent metabolic signatures in control and AD subjects differ with sex. Identification of the enzymatic pathways affected in AD could provide a foundation for therapeutic interventions. Comparison of metabolic alterations in peripheral cells, CSF and plasma Sex effect on metabolic signatures in AD and healthy individuals

POSTER 121

The Soluble Epoxide Hydrolase "Omics-Trio" Revealed Potential Treatment Targets for Anorexia Nervosa

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Anorexia nervosa (AN) is characterized by severe restrictive eating and emaciation, with high rates of morbidity, chronicity and mortality. Current treatments are ineffective and have a high rate of relapse, thus developing new treatments is a high priority in mental and public health. A limiting factor in developing new effective treatments is a lack of adequate knowledge on how molecular mechanisms of disease genes affect pathophysiology. We have taken an integrative multi-domain Omics (genomic, proteomic, lipidomic and metabolomics) research approach to investigate how molecular mechanisms of an AN susceptibility gene, soluble Epoxide Hydrolase 2 (EPHX2), that was discovered through our GWAS and exon sequencing studies, effects AN. Lipidomic and metabolomic assays were conducted using the GC/MS and LC/MS/MS systems. Quantitative profiling of polyunsaturated fatty acids (PUFAs), the parental substrates of soluble epoxide hydrolase (sEH), was done using plasma of 60 female AN patients and 36 healthy control women. Targeted oxylipin profiling was performed in 20 patients and 38 controls. Additional fasting and postprandial oxylipins were obtained in 6 patients and 5 controls to assess intraindividual oxylipin shift. Ex vivo sEH activity was directly measured in the buffy coats of 15 patients and 5 controls. Diol:epoxide oxylipin ratios and ω6:ω3 PUFA ratios were calculated as proxy markers of in vivo sEH activity and dietary PUFA markers, respectively. All statistical analyses were performed in R.3.1.3. Both the short-chain (LA:ALA) and long-chain (ARA:EPA) ω6 to ω3 PUFA ratios were lower in patients compared to controls (127 versus 196, $p<0.001$ for LA:ALA and 8 versus 17, $p<0.0001$ for ARA:EPA). Cytochrome P450 metabolites of PUFAs were associated with AN and showed a pattern of higher diol:epoxide oxylipin ratios in AN patients, suggesting that in vivo sEH activity is elevated in patients compared to controls. The direct ex vivo sEH activity measurement demonstrated higher activity in patients compared to controls (0.012 versus 0.007 nmol.min-1.mg-1, $p=0.05$), which is consistent with the data of sEH activity proxy markers. PUFAs are upstream parental substrates of sEH, therefore the dysregulation of PUFA profile observed in patients may play a role in the sEH-associated AN risk. To account for substrate-mediated effect and to explore metabolic consequences of sEH activity in AN, postprandial oxylipins that are substrates (epoxy-fatty acids) of sEH and the resulting metabolites (diol-fatty acids) were assessed and compared in patients and controls. After accounting for the potential confounding effects of pre-catalyst epoxy-fatty acid level (5.6.EET) and age, the postprandial shift of pro-inflammatory diol-fatty acid of ω-6 ARA, 5.6.DiHET, was more than 3-times higher the level observed in controls (1.51 versus -4.09, $p=0.09$). Interestingly, the diol-fatty acids from ω-3 ALA and DHA did not show significant difference between groups. The use of multi-domain EPHX2 omics markers reveals that upregulated sEH activity and resulting pro-inflammatory shift may be the key mechanism by which EPHX2 effects AN risk. Furthermore, dietary factor may modulate the deleterious effect sEH plays on AN risk, illness course, and outcome. This study is timely because both enzyme inhibition and dietary modulation are accessible approaches for AN treatment development.

POSTER 122

Metabolomic profiling of cerebrospinal fluid samples for atypical Alzheimer patients identification

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Currently, the diagnosis of Alzheimer's disease (AD) is established by clinical and neuropsychological evaluations. However, some clinical variant of AD don't meet the typical presentation and resorting to biomarkers of the cerebrospinal fluid (CSF) could be helpful. Although CSF biochemical profiles determinated by amyloid-β42 (Aβ42), total and phosphorylated tau (t-tau and Poster tau) concentrations are well established for AD, there's still a need to identify atypical presentations, such as primary progressive nonfluent aphasia (PPA) and posterior cortical atrophy (PCA). CSF metabolomic exploration would allow the identification and quantification of specific metabolites for these diseases. Our aim is to study CSF metabolomic profiles associated with atypical AD in order to classify these different syndromes, allowing better patient care and better physiopathology understanding Currently, the diagnosis of Alzheimer's disease (AD) is established by clinical and neuropsychological evaluations. However, some clinical variant of AD don't meet the typical presentation and resorting to biomarkers of the cerebrospinal fluid (CSF) could be helpful. Although CSF biochemical profiles determinated by amyloid-β42 (Aβ42), total and phosphorylated tau (t-tau and Poster tau) concentrations are well established for AD, there's still a need to identify atypical presentations, such as primary progressive nonfluent aphasia (PPA) and posterior cortical atrophy (PCA). CSF metabolomic exploration would allow the identification and quantification of specific metabolites for these diseases. Our aim is to study CSF metabolomic profiles associated with atypical AD in order to classify these different syndromes, allowing better patient care and better physiopathology understanding All samples were analysed using C-18 and HILIC LC coupled with ESI+ and ESI- LC-HRMS. All raw data (QCs and patients) were firstly pre-processed using Sieve software for alignment and features detections. Each frame was inspected manually and frames that corresponded to background noise were excluded. QC samples issued from a pooled mixture of equal volumes of all samples were analysed to assess reproducibility of the method. The stability of mass accuracy and retention time were checked. QCs were evaluated by principal component analysis and showed a tight cluster for all column types and ionization modes. Data were analysed by OPLS-DA analysis using SIMCA. Although no models were found to properly be able to discriminate APP and PCA patients from AD patients, APP and PCA patients were found to display different metabolic signatures. Best models were obtained after UV scaling data normalization. From the predictive variation between X (metabolites) given by R2X(cum), best models interpreted approximately 31, 71, 81 and 60% of the total variation in X for C18-ESI+, C18-ESI-, HILIC-ESI+ and HILIC-ESI- respectively. We found good predictabilities of the models given by Q2(cum) for biological data: 0.74, 0.83, 0.81 and 0.85 for C18-ESI+, C18-ESI-, HILIC-ESI+ and HILIC-ESI- respectively. While these models appear accurate, they need improvement, especially through the enrolment of larger cohorts of patients. This preliminary work reveals that APP and PCA patients seem to have different LCR metabotypes that would provide a differential diagnosis of these diseases, thus bringing arguments to help the diagnosis of atypical AD. Identification of several metabolites that discriminate the different profiles is in process. Their identification would give information on the pathophysiological pathways involved in these pathologies. We are currently enrolling patients in an independent cohort in order to better explore atypical AD patients. Metabolomics highlight molecular mechanisms potentially usable as pathophysiological and/or diagnostical biomarkers for atypical AD such as PPA and PCA

POSTER 123

Brain metabolome exploration by LC-HRMS in an ASD autism rat model

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Autism spectrum disorders (ASD) are a group of behaviorally neurodevelopmental disorders with lifelong consequences including pervasive impairments in social interactions, deficits in communication, and stereotyped, repetitive patterns of behaviors and interests. To date, ASD physiopathology(ies) remains largely unexplained and no biomarker is available for clinical purposes. In this context, we chose the mostly useda validated rodent model of autism that consists in exposure of rat fetuses to valproic acid on the 12th day of gestation in order to explore the developmental modifications in the brain metabolome associated with this disorder. Targeted and untargeted analyses of brain regions were achieved by LC-HRMS during the brain neurodevelopment in order to focus on metabolism pathways described as potentially altered in human ASD. Pregnant Wistar rats received an intraperitoneal injection of VAP (600 mg/kg) or vehicle (VHC) at GD12. Experiments were conducted on the male progeny at P21 (n=10) and P35 (n=8). Animals were sacrificed by decapitation, and hippocampus, striatum, cerebellum, frontal and parietal cortices dissected. The metabolome extraction methodology from tissues was validated. After lyophilisation, metabolites were extracted with acetonitrile/water (1:1) and the supernatant was evaporated. Residues were dissolved in methanol/water (1:1) and analysed by LC coupled to high-resolution mass spectrometry (LC-HRMS) in both positive and negative ionizations modes. Targeted analyses used an in-house library containing 136 metabolites form classical metabolism pathways (i.e. amino acids, neurotransmitters, vitamins, purines-pyrimidines). Untargeted analyses used XCMS and SIMCA software for important features extraction and identification. We evaluated the tissue extraction methodology for targeted and untargeted metabolome screening, using several masses of brain tissue (3, 6 and 9 mg of brain). For each condition, after signal normalization to total area and reduction of detected features with CV>30%, no significant differences of the number of detected features were obtained for 3, 6 and 9 mg. Among these features, 136 metabolites were identified by comparison with our library, belonging to amino acids, neurotransmitters, organic acids and other metabolites from pathways described to be altered in ASD. The methodology characterization was carried on using 3 mg of brain tissue, giving us the opportunity to explore specific small brain regions. In order to evaluate the sample extract stability over 36 h, multi injections at t=0, 12, 24 and 36 h wereas realized. Among the 1872 and 908 detected frames in ESI+ and ESI-, 94% (1755) and 97% (885) of features showed less than 30% of signal variability over 36 h. When focused on identified metabolites, only 28 of the 136 metabolites showed CV values >30% when samples werare analysed at 36 h interval. The high mass resolution (R=70 000) resulted in accurate masses with deviations lower than 5 ppm (n=7). Intraday reproducibility was evaluated by extraction of the same lyophilized tissue (n=4). Untargeted analyses showed average CVs of 11 and 13% on the detected frames in ESI+ and ESI-. Regarding the targeted analysis, 82% of identified metabolites showed less than 30 % of signal variability. This extraction methodology gives a satisfying robustness for brain metabolome exploration by LC-HRMS. We are currently analysing several brain regions at two critical stages of neurodevelopement (P21 and P35) in order to identify metabolic pathways implied inmvolved in normal neurodevelopment, but also those linked to the three cores signs of ASD expressed in VPA condition. Metabolome and brain neurodevelopment in an autism rat model

POSTER 124

Metabolomics-based Profiling of Blood Plasma in Children with Autism Spectrum Disorder (ASD): A Search for Discriminatory Metabolic Subtypes

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Autism spectrum disorder (ASD) is a complex and poorly understood condition thought to involve different genetic, metabolic and environmental factors among individuals. Research into the metabolic etiology of this complex disorder can identify metabolites related to ASD providing insight into the cause, leading to more reliable diagnosis and treatments. Herein we report on an untargeted metabolomics approach to profile the blood from Typically Developing (TD) and ASD children enrolled in the Autism Phenome Project (APP). Our goal was to discover biomarkers that can be used for predictive, diagnostic metabolic signatures across the complex spectrum of the disorder. In addition, we illustrate how an analysis of certain features can suggest the existence of metabolic subtypes among individuals with ASD. Plasma was obtained from 180 children (ages 2 to 4) with ASD and from 93 age-matched TD children enrolled in the APP. Samples were analyzed using 4 orthogonal HILIC and C8 LC/HRMS-based methods as well as GC/MS. Data from the patient samples were split into a training set, utilized for discovery profiling, and an independent validation test set used for evaluation of the diagnostic signatures. Univariate, heuristic, multivariate, machine learning and class discovery methods were applied to the training set to identify metabolic features that are capable of classifying patient plasma samples as being from ASD or TD children and/or differentiating metabolic subtypes among ASD individuals. The molecular signatures were evaluated in the validation test set to determine their performance. The untargeted metabolomics approach successfully identified metabolite signatures which discriminate individuals with ASD from TD subjects. Univariate and heuristic analysis of LC-MS features identified 1608 mass features (25% of the feature set) passing quality control criteria for abundance and peak shape. Computational models were created using subsets of these features that could classify the ASD and TD samples in the independent validation test with a preliminary maximum accuracy of 81% and AUC of 0.82. Evaluation of the statistically significant metabolic features (p value 10 fold, p value < 1e-6) in subset of ~20% of ASD patients as compared to other ASD and TD individuals. The differential abundance of CMPF and its correlated features suggests a possible metabolic subtype of ASD. Further combinations of highly discriminatory features are being evaluated for use in metabolic subtyping and identification of patients with ASD. Applying a paradigm such as this to identify metabolic signatures associated with ASD and elucidating their biochemical implications may be useful in directing therapy on personalized basis. Broad based discovery metabolomic profiling of children with ASD reveals predictive metabolic signatures and suggests metabolic subtypes

POSTER 125

A metabolite profile and implications for disease mechanisms from a mouse model of Charcot-Marie-Tooth type 2D

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Charcot-Marie-Tooth (CMT) disease encompasses a genetically heterogeneous class of heritable motor and sensory neuropathies that result in axonal degeneration in the peripheral nervous system. Charcot-Marie-Tooth type 2D (CMT2D) is caused by dominant mutations in Glycyl tRNA Synthetase (GARS), and mutations in the mouse Gars gene result in a genetically and phenotypically valid animal model of CMT2D although how it leads to peripheral neuropathy is unclear. A profile of altered metabolites that

distinguish the samples from the spinal cord of GARS mutant mice and litter-mate control animals was determined. This study improves our understanding of changes associated with GARS mutations and suggests possible treatment strategies through supplementation, but an understanding of the disease mechanism will require additional comparative analyses. Spinal cord and sciatic nerve tissues were collected from six week old GarsNmf249/+ and Gars+/+ litter-mate mice of both sexes housed at The Jackson Laboratory under standard conditions. The samples (spinal cord: 50-100 mg per animal, sciatic nerve: 5-10 mg per animal) were snap frozen in Eppendorf tubes in liquid nitrogen and subsequently analyzed using UHPLC-MS/MS ± ESI and GC-MS+EI. Spinal cords were analyzed individually, whereas sciatic nerves were combined into one mutant and one control pool to have sufficient tissue for analysis. Data from 20 samples (12 Control/10 Mutant) with a total of 289 metabolic features (228 structurally known) was analyzed using exploratory, univariate, multivariate and machine learning methods. Serum ascorbate levels were assayed using a colormetric detection system. Following mass spec analysis of metabolites, various exploratory and multivariate analyses including heat map, hierarchical clustering analysis and principal component analysis (PCA) indicated clear separation between the two genotypes. The Receiver operating characteristic (ROC) curve derived from averaging the performance of 50 re-sampled iterations of Support Vector Machine (SVM) classifier on the test samples showed robust discrimination between the affected and unaffected tissues (AUC = 1) as compared to the 50 re-sampled iterations of the SVM classifier on samples with reshuffled class labels (AUC= 0.45, 95%CI 0.39-0.51).In the t-test analysis, 112 features showed statistically significant changes between the two genotypes (Poster value <=0.05). Ascorbate and carnitine were significantly down regulated in the mutant samples (ascorbate 70% down, FDR = 0.002, carnitine 30% down, FDR= 1.31E-07) and were also marked as top features in the SVM analysis. Ascorbate levels were unaffected in mutant versus control mouse serum, suggesting this change is specific to disease-relevant tissue and not a secondary systemic or dietary change. Levels of several metabolites in the cholesterol and neurotransmitter biosynthetic pathways were affected. In addition, all metabolites associated in the urea cycle were elevated in the GarsNmf249/+ samples, suggesting potential candidates for bio-marker discovery. Serum assayed from an independent mutant (Agrn) with similar neuromuscular phenotype showed that changes were specific to the GarsNmf249/+ mutant mice and not secondary to impaired neuromuscular performance. The metabolites with the largest magnitude decrease in the mutant samples are potential targets for therapy through dietary supplementation. Recent clinical trials have been testing ascorbate supplementation as a treatment for demyelinating CMT Type 1A (CMT1A) based on success in transgenic animal models overexpressing Pmp22, the genetic cause of CMT1A. Carnitine is also suggested to be beneficial in a variety of neurological settings, including the regeneration of peripheral axons after injury. This a first metabolomic fingerprint from a CMT2D mouse model for comparisons with other neuromuscular mutations to explore disease mechanisms.

POSTER 127

Mass spectrometry analysis of alpha-Galactosidase A metabolites, globotriaosylceramide and globotriaosylsphingosine, in Parkinson disease brain tissues.

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Parkinson disease (PD) is characterized by the presence of Lewy bodies containing aggregates of alpha-synuclein in brain tissues. Our preliminary data indicate that alpha-Galactosidase A (alpha-Gal A) deficient mice present pronounced accumulation of alpha-synuclein in several brain regions, suggesting that alpha-Gal A regulates alpha-synuclein metabolism. The main function of the alpha-Gal A enzyme is to cleave the last sugar unit of globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3). Alpha-Gal A deficiency is also the cause of Fabry disease, an X-linked lysosomal storage disorder. Metabolomic studies performed in our laboratory have revealed Gb3 isoforms/analogues, and lyso-Gb3/related analogues as Fabry disease biomarkers in urine, plasma, and tissues. This study is oriented towards the evaluation of these molecules as possible PD brain biomarkers. For this research study, 12 brain tissue specimens from PD patients (Stage 3) and 12 brain tissue specimens from healthy controls

were provided through a consortium affiliated with the Michael J. Fox Foundation. Each brain tissue sample (100 mg) was homogenized in methanol with a Bead Ruptor 12 (Omni International) to obtain a concentration of 0.1 mg/mL. For the analysis of Gb3 isoforms/analogues, 200 µL of brain homogenate was purified by liquid-liquid extraction with chloroform. For the analysis of lyso-Gb3 and related analogues, 100 µL of brain homogenate was purified by solid phase extraction using a mixed-mode cation-exchange cartridge (Oasis, Waters). Samples were analyzed by ultra-high performance liquid chromatography (UPLC, Acquity, Waters) hyphenated to tandem mass spectrometry (Xevo TQ-S, Waters). A multiple reaction monitoring (MRM) method was developed and optimized for the analysis of lyso-Gb3 and 8 related analogues presenting modifications on the sphingosine chain in brain samples of PD patients and controls. Glucopsychosine, a sphingolipid structurally related to lyso-Gb3, but present only in plants, was used as the internal standard. For the method validation, 40 pg of lyso-Gb3 /g brain were spiked in brain homogenates from pooled healthy controls. For the intraday assays (n = 5), we obtained a relative standard deviation (RSD) of 18.6% and a bias of 5.2%. For the interday assays (n = 5), we obtained RSD and bias of 11.5% and 5.2%, respectively. The limits of detection (LOD) and of quantification (LOQ) were respectively 20.4 and 68.0 pmol/ g brain. Lyso-Gb3 and the 8 related analogues were not detected in the two sample groups analyzed. A second MRM method was developed and optimized for the analysis of 29 Gb3 isoforms/analogues in brain samples. Gb3(C18:0)D3 was used as the internal standard. For the method validation, a commercial mixture of Gb3 containing 7 isoforms/analogues (Matreya) was spiked to brain samples from pooled healthy controls. The concentration of the 7 isoforms/analogues ranged from 0.16 to 3.15 µg/ g brain. For the intraday assays (n = 5), we obtained RSDs <12% and a bias <9% and for the interday assays (n = 5), we obtained RSDs <12% and bias <15%. The LOD and LOQ were <0.025 and <0.085 µg/ g brain, respectively. The three most abundant Gb3 isoforms, Gb3(C16:0), Gb3(C16:1), and Gb3(C18:1), were slightly more abundant in the PD group compared to the control group. However, a study on a larger cohort of PD patients and controls will be necessary to confirm a statistically significant difference between the two groups. This study reveals relative quantification of 29 globotriaosylceramide/isoforms-analogues, and globotriaosylsphingosine/8 related analogues in brain tissue specimens from Parkinson disease patients.

POSTER 130

Ultra high resolution mass spectrometry-based metabolic characterization reveals cerebellum as a disturbed region in two animal models

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Cerebellum was thought to be a less affected tissue by genetic or environmental alterations in comparison to other tissues in the brain including hippocampus under the same conditions. In this work, we investigated two types of metabolomic alterations inside the cerebellum tissue. The first one addressed the differences in the metabolomics profiles between Transgenic (Tg) CRND8 of Alzheimer's disease mice and non-transgenic (non-Tg) littermates. The second one addressed the metabolic differences between wild type mice exposed to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) and wild type mice which are not exposed to this toxic compound. Ultra high resolution Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR/MS) was implemented. Significant changes of each comparison were tentatively annotated by the high mass accuracy generated from the measurements in the negative ion mode. The biosynthesis of amino acids was enhanced pronouncedly, and perturbation of purine metabolism was also observed in Tg mice compared to non-Tg littermates. In another animal model, the reduced levels of amino acids were found where as the intermediate levels in purine metabolism and fatty acids including fatty acid conjugated metabolites were elevated in cerebellar tissues of mice exposed to TCDD compared to control group. Collectively, it was demonstrated that FT-ICR/MS was a powerful tool for interpretation of the elemental compositions of the peaks, revealing that the metabolic perturbations in cerebellar tissues of mice were induced by either genetic manipulation or environmental factor. Therefore, the non-targeted approach,alternatively, provides various metabolic phenotypes for the systems-level mirror of the complex etiology of neurotoxicity in the cerebellum. This work provided a method to assay the metabolomic changes of the cerebellum (Talanta 118 (2014) 45–53.).

POSTER 131

Metabolic investigation of epilepsy and seizure etiology for the discovery of novel therapeutic agents

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Epilepsy is a disorder which effects up to 2% of the population and is characterized by recurring unprovoked seizures. While the majority of cases are idiopathic, evidence from better studied forms of epilepsy indicate a critical metabolic component. The ketogenic diet (KD), is effective in the treatment of epilepsy, but interestingly not limited in scope to those epilepsies caused by defects in metabolic related genes, such as Glut-1. For this reason we undertook an investigation of epilepsy by treating the disease as a metabolic disorder and through both targeted and non-targeted metabolic approaches looked to define the metabolic background of epilepsy as a means to identify novel natural metabolites which may serve as anti-seizure and thus anti-epileptic therapies. Tissue used included surgical resections from patients with epilepsy as well as a Kv1.1 KO mouse model of familial epilepsy. Non targeted lipid analysis was carried out on a Bruker Solarix FT-ICR by LC/MS. Amino acid analysis was performed using the aTRAQ method on an ABSciex 4000 Q-Trap. Proteome screening for oxidative modification (carbonylation) was performed using 2-D DIGE followed by peptide analysis on a Thermo Velos Pro LTQ. Adenosine energy metabolites were determined through etheno adduct formation and fluorescent detection by HPLC. A C. elegans model for seizures was developed using RNAi of various genes in combination with drug treatment (pentylenetetrazole (PTZ)). Selected compounds were screened on the ability to rescue worms from seizures via mobility assays. Untargeted lipid analysis of mouse Kv1.1 KO model vs. WT cortex showed minimal change, but of the 2,952 peaks identified from the hippocampus 44 showed a significant change (2 fold, $p<0.05$). When comparing human seizure onset zone tissue to quiet zone tissue 237 out of 13,288 identified features had a significant change. Features identified in both the mouse model and human tissues are largely phospholipids suggesting dysregulation of phospholipid metabolism. Adenosine energy metabolites identified in the mouse model showed in both the WT mouse cortex and hippocampus ATP/ADP/AMP ratio is stable, while Kv1.1 KO mouse have widely varying ratios, indicating disruption of energy homeostasis. Approved sample collection protocol of human brain tissue prevented adenosine analysis. Amino acid analysis of human tissue showed no significant change between quiet zone, onset zone, and irritative zone (irregular spiking on EEG but not seizure) for all amino acids analyzed with the exception of serine, where we saw a significant decrease of serine in irritative zone. We hypothesize that this depletion hypersensitizes NMDA receptors in tissue adjacent to seizure onset zones, thus allowing the sudden spike in activity which is associated with epilepsy, and indeed, D-serine has been pursed as a treatment for epilepsy. In addition, serine biosynthesis depends on activity of glycolysis enzymes suggesting imbalance of this key energy pathway. Finally, we observed increased oxidation of key glycolytic enzymes glyceraldehyde-3-phosphate dehydrogenase, triosphosphate isomerase, and phosphoglycerate mutase in onset tissue compared to quiet zone tissue, indicating defects in glycolytic enzymes which depletes energy and disrupts redox homeostasis in epileptic tissue. To investigate the role the metabolites identified, and products of enzymes identified, play in seizure, we have to date developed two C. elegans models which produce seizure like movements and impaired mobility towards food upon treatment with PTZ; RNAi KD of either Lis-1 or Bas-1. Identification of metabolites involved in epilepsy will provide novel and more tractable therapeutic avenues for the treatment of epilepsy.

POSTER 132

Understanding the role of pyridoxal 5'-phosphate in pyridoxine-dependent epilepsy: The metabolic profiling of antiquitin deficiency.

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Inborn errors of lysine metabolism can result in a rare class of devastating intractable disease known as pyridoxine-dependent epilepsy (PDE). Autosomal recessive mutation of ALDH7A1, the gene encoding antiquitin (i.e. aldehyde dehydrogenase) leads to accumulation of the lysine degradation intermediate α-aminoadipic semialdehyde, which is thought to trap the essential cofactor pyridoxal 5'-phosphate through Knoevenagel condensation. Diminished PLP results in myriad secondary pathomechanisms due to perturbations in neurotransmitter, amino acid and one-carbon metabolism. Here we employ global metabolic profiling to better understand the secondary pathomechanisms of antiquitin deficiency through identification of the metabolic pathways most heavily affect by the disease. The results of this work may lead to better long-term therapy and PDE patient outcome. Cerebro-spinal fluid (CSF) was obtained by lumbar puncture. Plasma samples were isolated by centrifugation from the whole blood of patients with genetically confirmed antiquitin deficiency. Patient-derived fibroblasts were obtained from skin biopsy by established methods. Metabolites in the culture medium were controlled by the use of depleted media, with the addition of labeled substrates. All metabolomics data was collected by liquid chromatography – high resolution, accurate mass spectrometry (LC-HRAMS). HILIC- and C18 stationary phases were utilized for separations running gradients of acetonitrile and 5mM ammonium formate, at pH 3. Data were collected in both positive and negative-mode electrospray ionization modes. Statistical analysis included Kruskal–Wallis one-way analysis of variance, PCA, OLPS-PA and ROC. Analysis of the untargeted LC-HRAMS data from 9 patient biofluid samples (6 plasma; 3 CSF) and experiments on antiquitin deficient patient-derived fibroblast cell lines demonstrates the diversity of metabolic pathways affected by a deficiency of the enzyme antiquitin. In the CSF data for example, 5905 total features were aligned by XCMS. Of these, 366 were confidently identified as being variably expressed between the experimental and control cohorts. Pathway analysis of these metabolites indicates heavy perturbation in the metabolism of the amino acids tryptophan, arginine and proline. While a variety of additional pathways are indeed affected, these preliminary data already help delineate the complex relationships of antiquitin, PLP and the severe epileptic phenotype. To our knowledge, this is the first report of the metabolomics analysis of antiquitin deficiency and pyridoxine-dependent epilepsy.

POSTER 133

In situ metabolomic signatures of neuronal survival, apoptotic progression, and microglial phagocytosis

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Metabolic dysregulation is an early hallmark of neurodegenerative diseases including Alzheimer's disease and age-related macular degeneration. Mapping metabolic adaptation with cellular resolution and tissue-wide context is crucial to define networks regulating neuronal survival, cell death progression, and immune cell response. Computational Molecular Phenotyping (CMP) explores the amine metabolome (amino acids and amines). Technically, CMP metabolomics combines amine metabolite trapping, ultrathin microscopy (50-200 nm), immunodetection, pattern recognition, and clustering algorithms. Here we mapped the in situ distribution of over 30 core amine metabolites in retinal cells challenged by light-induced oxidative stress. Metabolomic profiles were phenotyped using ultrastructural, biochemical, and proteomic indices of oxidative stress. CMP enabled precise visualization of >30 metabolites in every retinal cell. CMP resolved and phenotyped metabolomic profiles to specific degeneration and microglial functional states in the light-damaged retina. Cone photoreceptor survival correlated with enhanced

antioxidant glutathione content. Rod photoreceptor apoptosis coincided with rapid depletion of organic osmolytes followed by nuclear import of cationic arginine metabolites. Delay in cell death increased necrosis and DNA damage-induced apoptosis. Microglial chemotaxis enhanced distinct signatures of glutamate and glutathione metabolism; whereas, phagocytosis coinduced classic (M1) and alternative (M2) arginine metabolites of macrophage activation. CMP discovers and phenotypes cell classes, tracks cell state, and maps disease with single-cell resolution in any tissue or organism.

POSTER 134

Identification of blood-based metabolic markers of severity and patient outcomes in traumatic brain injury

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Traumatic brain injury (TBI) is a common cause of neurological damage and disability. It is a major cause of death and disability worldwide. TBI is usually graded as mild, moderate, or severe on the basis of the level of consciousness, assessed using the Glasgow Coma Scale. Small molecules are more sensitive to changes in blood brain barrier (BBB) functionality than larger molecules, and thus more likely to pass the BBB following an injury. Here we applied comprehensive metabolic profiling of serum samples from TBI patients and controls in two independent studies. We also studied brain microdialysate samples from selected patients, in order to further confirm the relevance of observed serum metabolic profile in TBI to altered metabolism in the brain. The study subjects comprised of consecutive non-selected subjects with acute TBI. The control group comprised of acute orthopaedic non-brain injuries. Two separate sample cohorts were utilized in this study (Turku and Cambridge), in total comprising 207 TBI cases, divided into mild, moderate and severe, and 54 controls. The blood samples were taken directly after hospitalization, and then after 1, 2, 3 and 7 days after the accident. The health status of the patients, including medications in use and possible use of drugs or alcohol was recorded for each patient. The established metabolomics platform based on two-dimensional gas chromatography coupled to time-of-flight mass spectrometry (GCxGC-TOFMS) was applied to analyze the plasma samples. Data were processed using the Guineu software. A total of 851 metabolites were measured, including amino acids, sugar derivatives, hydroxyl acids, fatty acids and sterols and related metabolites. In the arrival samples, 152 metabolites showed significant differences between the TBI patients and controls in the Turku cohort (FDR $q<0.05$), and 110 metabolites in the Cambridge cohort. 39 metabolites were significant in both of the datasets. The differences between cases and controls were most pronounced for the severe TBI patients, while the differences were much smaller in the mild or moderate TBI patients. Most of the compounds were significantly upregulated. Specifically, two medium-chain fatty acids, octanoic acid (OA) and decanoic acid (DA), were significantly upregulated in severe TBI. Some metabolites, such as several amino acids including branched chain amino acids (BCAAs) were downregulated. No significant differences were observed between the arrival samples of mild TBI and control groups when the two sample cohorts were studied separately. However, when the two sample cohorts were combined, significant differences could be detected between the controls and (mild and moderate) TBI patients with significantly upregulated sugar derivatives and oxygenated short-chain carboxylic acids, including lactic acid. The metabolites found associated with severity of TBI were also predictive of patient outcomes following the TBI. In order to study the potential relevance of the serum metabolic profiles in TBI to brain metabolism, brain microdialysates (BMD) were analyzed from selected TBI patients (Cambridge cohort). In addition, the metabolites were searched from CSF samples by the same analytical methodology. Some of the top serum metabolites associated with TBI were also present at high concentrations in BMD and CSF.

Notably, the medium-chain FAs (C7-C10) including OA and DA were detected at relatively high concentrations in BMD as compared to their corresponding concentrations in blood, while the long-chain FAs had clearly lower levels in BMD than in blood. The metabolites found associated with TBI may serve as diagnostic, predictive and patient monitoring markers of TBI in healthcare setting.

POSTER 135

Metabolomics reveals new pathways and candidate biomarkers of vitamin B12 status and effects of B12 on myelinated peripheral nerve conduction

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Vitamin B12 deficient asymptomatic Chilean elderly displayed improved conductivity of myelinated peripheral nerves after vitamin B12 treatment. The objective of this study was to identify metabolic pathways and candidate biomarkers that connect vitamin B12 status to myelinated peripheral nerve conduction. Targeted [166 carnitines, aminoacids, sugars, glycerophospholipids and sphingolipids (BIOCRATES Absolute IDQTM)] and untargeted [247 (109 unknown) primary metabolites (GC-TOF MS)] metabolomics were measured in serum samples of Chilean vitamin B12 deficient elderly before (n=27) and after (n=27) 4 months of receiving treatment (single i.m injection 10 mg cyanocobalamin), and in a sub-sample of elderly with adequate vitamin B12 status (n=18). Vitamin B12 status was determined and sensory nerve conduction studies were performed (Nicolet Viking Quest TM system, Nicolet Biomedical, Madison, WI, USA). Merging the three sub-groups into a heat map correlation matrix indicates direct ($p<0.0001$) correlations between vitamin B12 status (based on total plasma B12, holo transcobalamin, total plasma homocysteine, methylmalonic acid and the combined indicator of vitamin B12 status) and a large family of plasmalogens, phospholipids, sphingomyelins and carnitines. Proline was inversely ($p<0.0001$) correlated with vitamin B12 status. The same metabolomics profile of vitamin B12 status was related to peripheral nerve function (latency right sural nerve). Changes pre-post treatment show increases ($p<0.05$) in plasmalogens, phospholipids and carnitines and reductions in one carbon metabolism moieties (i.e. methionine, cysteine and cysteine). Metabolomics reveals new pathways and candidate biomarkers of vitamin B12 status and effects of B12 on myelinated peripheral nerve conduction

POSTER 136

Identifying blood metabolite modules associated with Alzheimer's Disease and assessing causality

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The need to understand the exact biological mechanisms underlying Alzheimer's Disease (AD) in order to diagnose it early and develop novel effective treatments has never been more urgent. With the estimated

worldwide number of patients suffering from dementia rising to upto 115.4 million in 2050, AD is undoubtedly one of the major healthcare challenges of the 21st century. Recently, a number of non-targeted blood metabolomic studies have been performed highlighting the role of lipid compounds in AD disease initiation and progression. The aim of this study was to use a systems biology approach to identify clusters of metabolites, likely to posses common function and correlate them to AD and to assess their causal association. A sample of >300 AD patients and healthy elderly controls was used. We performed a comprehensive untargeted lipidomic analysis, using Ultra-Performance Liquid Chromatography/Mass Spectrometry generating features (>600 features) and a Genome Wide Association (GWA) study followed by imputation (>6 million SNPs). Weighted Gene Co-Expression Network Analysis (WGCNA) was used to identify clusters (modules) of metabolites. Linear and logistic regression analyses correlated the identified modules with AD as well as cognitive decline and brain atrophy (AD endophenotypes). Linear regression analysis was performed to identify genetic variants associated with the extracted modules (using SNPTEST) and Mendelian Randomisation was used to assess causality. Following stringent quality control for all datasets, twenty modules of metabolites were identified through WGCNA with six of them showing associations with AD and AD endophenotypes. Specifically, a module ("green") comprising mainly of lipid molecules showed associations with more that one AD phenotype. A number of genetic loci are associated at Bonferonni corrected Poster value with a number of modules. Results from the integration analysis and Mendelian Randomization will be presented. This is the first study to perform such integrative analysis in metabolomics and AD.

POSTER 139

Metabolomics of plasma reveals changes in the peripheral nervous system metabolism in Guillain-Barre syndrome

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Guillain-Barre syndrome (GBS) is a rare disease of acute inflammatory polyneuropathy affecting the demyelination in peripheral nervous system. And metabolomics is a growing field in systems biology and has been shown to be a powerful approach to measure the metabolic profiles of individuals in response to disease or treatment. We applied LC-MS based metabolomics approach to delineate the pathophysiological mechanism of GBS and to discover potential biomarkers for this disease. Data show that the plasma level of histidine, aspartate, taurine, acylcarnitines, lysophosphatidylcholines, lysophosphatidylethanolamines, phosphatidylcholines, cholesterol, and sphingomyelins were significantly lower in GBS patients compared to control group suggesting a disturbance of lipid metabolism in GBS patients. The metabolic profile also reveals that the plasma levels of purine metabolites containing adenine and hypoxanthine were significantly increased in GBS patients indicating a high energy demand in the myelin sheath of these patients. My results also show that the extraordinarily high level of lipid synthesis, energy consumption, and uptake of external lipids from circulating system were unique characteristics of GBS patients. Such unique lipid metabolic pattern may be potential biomarkers for the diagnosis of GBS.

POSTER 141

1H-NMR based metabolomic analysis of cerebrospinal fluid from adult bilateral moyamoya disease: comparison to unilateral moyamoya disease and atherosclerotic stenosis.

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Although metabolomics has been increasingly used to observe metabolic pattern and disease-specific metabolic markers, metabolite profiling for moyamoya disease (MMD) has not yet been done in

adults. This study was undertaken to reveal cerebrospinal fluid (CSF) metabolites which were specific to bilateral MMD compared to unilateral MMD or atherosclerotic stenosis using a 1H-NMR spectroscopy. CSF samples of bilateral MMD (n=29), unilateral MMD (n=11) and atherosclerotic cerebrovascular disease (ACVD) (n=8) were recruited. Principal Components Analysis (PCA), PLS-Discriminant Analysis (PLS-DA) and Orthogonal Projections to Latent Structure Discriminant Analysis (OPLS-DA) were done for the comparisons. Diagnostic performance was acquired by prediction of one third left-out samples from the distinction model constructed with the rest of the samples. Bilateral MMD showed an increase in glutamine ($p<0.001$) and taurine ($p=0.004$) and a decrease in glucose ($p<0.001$), citrate ($p=0.002$) and myo-inositol ($p=0.006$) than those in ACVD. Unilateral MMD showed a higher level of glutamine ($p=0.005$) and taurine ($p=0.034$) and a lower level of glutamate ($p<0.004$) than those in ACVD. No difference at the metabolite level was observed between bilateral and unilateral MMD. Cross validation with the OPLS-DA model showed a high accuracy for the prediction of MMD. This study suggests that elevated glutamine in the CSF may be associated with MMD pathogenesis which differed from ACVD.

POSTER 142

Time-dependent metabolomic profiling of Ketamine drug action reveals hippocampal pathway alterations and biomarker candidates

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At present most clinically used antidepressants are targeting monoaminergic reuptake mechanisms. However, a limited efficacy and a delayed onset of therapeutic response combined with several side effects make them less than ideal drugs. Approximately one third of patients are suffering from treatment resistant depression (TRD) and do not respond to commonly used antidepressants. To improve antidepressant drug efficacy, one line of research has focused on the glutamatergic system, especially on the N-methyl-D-aspartate receptor (NMDAR) and its signaling pathways. Ketamine blocks the NMDAR with profound effects on downstream signaling cascades. Commonly used monoamine reuptake inhibitors improve depressive symptoms after weeks of treatment whereas Ketamine results in a therapeutic effect within hours and is particularly effective in patients suffering from TRD. We have carried out a time-dependent targeted polar metabolomics profiling study to analyze metabolite level and metabolite ratio changes and their contributions to the antidepressant-like effect of Ketamine drug action. C57BL/6 wild-type mice were treated with Ketamine in a time-dependent manner and polar hippocampal metabolites extracted. Metabolite profiles were analyzed with a 5500 QTRAP LC-MS/MS system via selected reaction monitoring followed by Q3 peak area integration. A total of 254 endogenous water-soluble metabolites were detected for steady-state analyses and interrogated and interpreted using several statistical methods (Partial Least Squares-Discriminant Analysis (PLS-DA), Significance Analysis of Microarrays and metabolites (SAM), and pathway enrichment analyses). Alterations affecting the metabolome are a reflection of modified pathway activities in response to drug treatment. The aim of the present study was to identify pathway alterations and biomarker candidates of Ketamine drug action. The metabolite profiles separated Ketamine from control mice using multivariate PLS-DA for all time points. Our metabolomics data indicate time-dependent metabolite level and metabolite ratio alterations starting already after 2 h, reflecting the fast antidepressant effect of the drug. In silico pathway analyses revealed that several hippocampal pathways including glycolysis/gluconeogenesis, pentose phosphate pathway and citrate cycle are affected, apparent by changes not only in metabolite levels but also connected metabolite level ratios. We have for the first time shown that Ketamine not only impacts the citrate cycle, but also increases protein levels of the enzymatic subunit of the complex II of the oxidative phosphorylation (OXPHOS). Furthermore, Ketamine treatment affects GTP and NADH levels produced by the citrate cycle and ATP levels produced by OXPHOS complex V in a time-dependent manner. Taken together, our results show that a single injection of Ketamine impacts the major energy metabolism pathways. Finally, we were also interested in exploiting our metabolomics analysis data to identify a biosignature for the Ketamine drug response. Seven of the metabolites qualify as biomarkers. 2-

Ketoisovalerate, Glutathione, Maleate, Methylmalonate, SBP, Fumarate and Cytosine represent stable and consistent metabolite biomarkers for all the investigated time points. This study provides new insights into Ketamine's mode of action and pathways that are affected downstream of the NMDAR.

POSTER 143

Monitoring efficacy of mitochondria-targeted therapeutics for neurodegenerative diseases using metabolomics

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Development of therapeutic strategies to prevent Alzheimer's Disease (AD) is of great importance. We demonstrated that mild inhibition of mitochondrial complex I with small molecule inhibitors reduces levels of amyloid beta and phospho-Tau and averts cognitive decline in three animal models of AD. Low-mass molecular dynamics simulations and biochemical studies confirmed molecular mechanism that includes activation of AMP-activated protein kinase in neurons and mouse brain without inducing oxidative damage or inflammation. Modulation of complex I activity augmented mitochondrial bioenergetics increasing coupling efficiency of respiratory chain and neuronal resistance to stress. Metabolic signatures were obtained in plasma and brain tissue to establish whether this method could allow monitoring therapeutic efficacy providing translational blood-based biomarkers. Metabolic signatures were generated in plasma and brain tissue from age- and sex-matched non-transgenic and APP/PS1 mice treated for 2 months using GC-MS. The Agilent Fiehn GC/MS Metabolomics RTL Library was employed for metabolite identifications. GC-MS spectra were deconvoluted using AMDIS software, after that SpectConnect software was used to create metabolite peaks matrix. The matrix data were exported to SIMCA-P+ software for multivariate data analysis. Unsupervised principal component analysis was run to detect potential outliers. Supervised partial least squares discriminant analysis (PLS-DA) was performed to obtain additional information on differences in the metabolite composition of groups. PLS-DA models were calculated with unit variance scaling, and the results were visualized in the form of score plots to show the group clusters. Treatment of APP/PS1 mice induced shift in brain metabolomic profile indicating effectiveness of the drug and the ability to modulate brain metabolic state confirming the proposed molecular mechanism. Most important metabolites in group separation included molecules associated with improved mitochondrial function, increased neuroprotective AMP-signaling and activation of AMPK and metabolic enzymes; increased NAA levels indicated reversal of AD-related metabolic disturbances; increased levels of ascorbic/dehydroascorbic acids indicated improvement of vitamin C status and redox balance of the brain. Metabolomic profile in plasma differed form the one in the brain. Panel of most important metabolites in group separation (VIP) included Krebs cycle, amino acid and lipid metabolites with the largest weight for uracil and inorganic phospate. Decrease in L-tryptophan, tyrosine, alanine, palmitic and phosphoric acids in plasma of treated APP/PS1 mice may indicate better extraction and utilization of these metabolites. Increase in plasma fumarate, which is a mitochondrial biomarker, was unexpected, however it may reflect increased metabolic turnover of Krebs cycle, which is consistent with improvement of energy metabolism observed in brain extracts. Treatment of APP/PS1 mice with novel complex I inhibitor did not alter citrate synthase activity in the hippocampus, indicating preserved mitochondrial capacity and the absence of mitochondrial toxicity. We demonstrate that efficacy of novel therapeutics that protect against cognitive decline could be monitored in blood using metabolomics

POSTER 144

Urine Metabolomics Profile in Early CKD

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Urine is an easily obtained biological fluid that is useful for metabolomics analysis and holds promise for uncovering metabolic differences between normal and disease states, including chronic kidney disease (CKD). A conspicuous gap remains between the low sensitivity and retrospective nature of current clinical markers, our inability to predict those who will progress to end-stage kidney disease (ESRD), and the high prevalence of CKD in the U.S. (~26 million individuals). Patients with CKD typically have significant co-morbidities and high rates of cardiovascular mortality. Metabolomics may be informative to detect and intervene at early stages of CKD with great benefit to public health. Urinary metabolomics profiles of 30 African American-Diabetes Heart Study participants with Stages 2 and 3 CKD (eGFR=71.7±28.4ml/min/1.73m2; urine albumin:creatinine ratio [UACR]=478.5±774.2 mg/g; age=56.6±9.5 years; BMI=39.1±10.0 kg/m2) and 47 diabetic non-nephropathy controls (eGFR= 91.3 ± 20.4ml/min/1.73m2; UACR= 8.2 ± 6.4 mg/g; age=53.8±8.0 years; BMI=37.3±8.9 kg/m2) were compared. Data were generated using broad spectrum 1H NMR spectroscopy; the raw data binned and normalized to total integral of each spectrum to account for differences in urine concentration. Binned data were mean centered, pareto-scaled, and subjected to multivariate analysis, including principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA). Bins important for distinguishing cases and controls were identified using variable importance to projections (VIP) plots (VIP≥1) and library matched for metabolites. A specific set of metabolites differentiating diabetic individuals with CKD from those without CKD was identified: 1-methylhistidine, 3-methylhistidine, citrate, creatinine, dimethylamine, glutamate, glutamine, glycylproline, leucine, myo-inositol, N-phenylacetylglycine, o-phosphoserine, phenylalanine, proline, S-sulfocysteine, salicylurate, succinylacetone, and valine. All African American-Diabetes Heart Study participants have diabetes; cases and controls with long diabetes durations (≥14 years) were analyzed as a separate subgroup because diabetes duration is important in adjudicating diabetic nephropathy. The set of metabolites that differentiated cases from controls in this subgroup contained some previously identified metabolites (citrate, creatinine, dimethylamine, and valine) in addition to 2-methylglutarate, arginine, guanosine triphosphate, lysine, methylsuccinate, myo-inositol, N-acetyltyrosine, putrescene, and salicylurate. A subgroup of 40 patients was followed longitudinally for 3-5 years, and we were able to identify a distinct set of metabolites differentiating those patients who maintained stable kidney function (rate of eGFR decline<1ml/min/1.73m2/year) compared to individuals whose rate of eGFR declined >1ml/min/1.73m2/year: 2-oxoisocaproate, 3-hydroxybutyrate, 3-methyl-2-oxovalerate, 3-methylhistidine, formate, isoleucine, leucine, maltose, and methylsuccinate. Our study therefore defined three sets of urinary metabolites that associated distinctly with early CKD, diabetic CKD, and CKD progression. A number of these metabolites were amino acids (AA) and their derivatives, and altered serum AA profile has previously been associated with severity of non-diabetic kidney disease. Alterations in protein homeostasis are characteristic of advanced CKD, but it appears that alterations in AA homeostasis can be detected in early stages of CKD. Several metabolites imply changes in tricarboxylic acid cycle, consistent with the reported mitochondrial dysfunction in diabetic nephropathy. This study suggests that metabolomics may improve the detection of early kidney dysfunction from diabetes and may predict nephropathy progression.

POSTER 145

Interstitial Cystitis-Associated Noninvasive Biomarkers

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Interstitial cystitis/painful bladder syndrome (IC) is a chronic syndrome of unknown etiology that presents with bladder pain, urinary frequency, and urgency. The lack of specific biomarkers and a poor understanding of underlying molecular mechanisms present challenges for disease diagnosis and therapy. The goals of this study were to identify non-invasive biomarker candidates for IC and potentially gain new insight into disease mechanisms. In order to search for soluble metabolites that segregate with the diagnosis of IC, we have assembled a novel IC patient cohort (IRB#10-0751), and a nuclear magnetic resonance (NMR)-based global metabolomics analysis of urine from female IC patients and controls were performed. Principal component analysis (PCA) suggested that the urinary metabolome of IC and controls was clearly different, with 140 NMR peaks significantly altered in IC patients (FDR<0.05), compared to controls. Based on strong correlation scores, eight metabolite peaks were nominated as the strongest signature of IC. Among those signals that were higher in the IC groups, three peaks were annotated as tyramine, the pain-related neuromodulator. Two peaks were annotated as 2-oxoglutarate. Levels of tyramine and 2-oxoglutarate were significantly elevated in urine specimens of IC subjects, which were also confirmed by an independent mass spectrometry analysis. These preliminary findings suggest that analysis of urine metabolites has promise in biomarker development in the context of IC. These IC biomarkers hold the potential to shed tremendous light on a disease of which we know very little.

POSTER 146

Metabolomic Analysis of Duchenne Muscular Dystrophy

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Duchenne Muscular Dystrophy (DMD) is a fatal muscle degenerating disease caused by the absence of the dystrophin protein. Replacement of healthy muscle by lipid and fibrotic tissue results in boys with DMD succumbing to the disease in the third decade of life. Promising clinical trials are impeded, as current outcome measures lack therapeutic sensitivity and are susceptible to inherent limitations. As a result, there is need for sensitive and non-invasive biomarkers to track disease progression and prognosis in boys with DMD. In this study, we investigate the urine from boys with DMD using both NMR and MS. Metabolomics findings were then analyzed against a database of phenotype and genotype data for each boy including MRI, functional assessments, and medication logs. Urine was collected from boys with (n=54) and without (n=7) DMD ranging in age from 5–17. All metabolomics data was acquired by the Southeast Center for Integrated Metabolomics (SECIM), following protocols as defined on the Metabolomics Workbench. Urine 1D NOESY and 2D JRES, HSQC, and TOCSY spectra were acquired using a 600 MHz Bruker NMR spectrometer with cryogenic probe. Spectra were analyzed using in-house MATLAB scripts for quality control and analysis workflows. Targeted mass spectrometry was performed at Sanford Burnham. Both organic acids and amino acids were determined. Creatinine was quantified independently for each samples using UV detection. A database of phenotypic and genomic data was collected and maintained as part of the ImagaingDMD natural history trial. The major findings in this study were that the ratio of creatine to creatinine concentrations track with disease progression. In control urine, creatinine levels were 1.5 fold higher (p=0.001), with next to no creatine present (p<0.05). However, in the urine of boys with DMD, the Creatine/Creatinine levels were 4 fold higher that of control (p=0.01). These finding suggest an alternation of the creatine and creatinine regulation in DMD boys compared to controls. We then used the creatine/creatinine ratio to correlate to the MRI T2 measurements (index of disease progession). MRI T2 increases in damaged tissue compared to healthy tissue. We discovered that as the T2 increased, the creatine/creatinine ratio increased as well (R2 = 0.244, p=0.0001). This

gives insight into the state of the tissue, providing a diagnostic window into DMD. We then determined if the creatine/creatinine ratio changed with disease progression. Of the 18 longitudinal measurements (0.8 years ± 0.25 years), only in 10 creatine/creatinine increased, all of which had been on a consistent medication regimen. Although eight decreased, 3 had recently started on therapeutic levels of steroids and 3 others were on experimental medication regimens. These promising results show that the urine metabolic profile can be used to track disease progression in DMD and is sensitive to therapeutic changes. We are currently evaluating the data for additional metabolomic biomarkers of DMD disease progression. We correlated DMD metabolomics to a database of MRI, functional and genomic data,to track DMD progression.

POSTER 147

Untargeted metabolomics to reveal genotype-phenotype correlation in X-linked adrenoleukodystrophy

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Phenotype variability in inherited neurometabolic disease X-linked adrenoleukodystrophy (X-ALD) can be a matter of life and death ranging from benign adrenomyeloneuropathy (AMN) to severe fatal cerebral adrenoleukodystrophy (ALD). Primary gene defect (mutation/deletion in peroxisomal ABCD1) and the resulting accumulation of saturated very long chain fatty acids (VLCFA; C>22:0) in tissues (including brain) is common to all the phenotypes of X-ALD disease and hence, cannot predict the phenotype and disease course. We recently reported the loss of metabolic enzyme AMP-activated protein kinase (AMPK) in ALD patient-derived fibroblasts but not in AMN. AMPK is the “master regulator” of cellular metabolism and raises the exciting possibility that metabolic pathways and their products may be uniquely altered between AMN and ALD patients. Fibroblasts derived from healthy controls (CTL) and subjects with mild (AMN) and severe (ALD) forms of disease were cultured in DMEM with 15% FBS and antibiotic (pen/strep). After 24h cultures were supplemented with fresh media for 3hr. Cells (2x106) were snap frozen and stored at -80c until processed. Metabolites were extracted using Methanol:Chloroform:Water (8:1:1), reconstituted in methanol:H2O (1:1) and processed using Accurate-Mass Q-TOF LC/MS. 1290 Infinity Binary LC System (Agilent Technologies) was used for chromatographic separation and 6530 Accurate-Mass Q-TOF (Agilent Technologies) with a dual ASJ ESI ion source was used as the mass detector. Raw data processing was done using Agilent software (MassHunter Qual and ProFinder). Data analysis was performed in R (http://cran.r-project.org/) and by “MetaboAnalyst 2.5” (http://www.metaboanalyst.ca/). We profiled the global metabolome using liquid chromatography coupled with mass spectrometry to identify the relative levels of metabolites in patient-derived fibroblasts from healthy control (CTL, N=6), mild (AMN, N=6) and severe (ALD, N=6) disease. Quality of analysis was accessed by visual inspection of the chromatographic traces (total ion chromatograms) and relative quantification of the internal standards. Quality control data suggest that the analysis method was stable and reproducible across all samples. There were 2972 peaks identified from the LC/MS+ spectra with 1587 detected in at least 75% of the samples. PLS-DA revealed a clear separation between CTL, AMN and ALD groups, indicating presence of unique metabolite profiles for the CTL, AMN and ALD groups. Analysis of variance per metabolite identified 194 biochemical peaks that differ between the three groups ($P<0.05$; FDR<0.35). Post-hoc t-tests determined that 97 of these were differentially altered between AMN and ALD patient-derived fibroblasts, indicating altered metabolomics profile in AMN and ALD phenotypes. Among these 97 altered biochemical peaks, 33 were mapped to specific metabolites. Pathway analysis in Metaboanalyst identified 5 pathways that were significantly enriched in this set of 33. These included glycerophospholipid metabolism ($P<0.00371$), cysteine and methionine metabolism ($P<0.0103$), pantothenate and CoA biosynthesis ($P<0.0203$), beta-Alanine metabolism ($P<0.0218$), glutathione metabolism ($P<0.0386$) and galactose metabolism ($P<0.0443$). Initial analysis, presented here, focuses on positive ion separation (LC/MS+). Future integration of the LC/MS- data, also available, will further enhance our understanding of the metabolic alterations across the spectrum of disease from Healthy to AMN to ALD. Gene defect and VLCFA accumulation are similar in X-ALD phenotypes. Metabolic alterations may serve as biomarkers and disease-modifying therapeutic targets.

POSTER 148

Pharmacometabolomics Identifies Acylcarnitines Associated With Beta Blocker Induced Fasting Glucose Alterations - Findings from RCMRC #U24DK097209

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B-blockers, commonly prescribed for hypertension treatment, are proven blood pressure lowering agents. However they are also associated with adverse metabolic effects, including hyperglycemia and diabetes. Knowledge regarding the mechanistic underpinnings of β-blocker-associated dysglycemia is incomplete. Identifying a biomarker that predicts the onset of dysglycemia accompanying b-blocker therapy could lead to a more personalized and safer approach for hypertension management. Acylcarnitines (ACs) are a large class of metabolites (derived from the corresponding acyl-CoAs) that signal the involvement of a host of different metabolic pathways. We used a targeted pharmacometabolomics approach focused on ACs to probe for a possible association between the baseline AC signature and dysglycemia after treatment with the commonly prescribed β-blocker, atenolol. Caucasian hypertensive patients (n=225) who were treated with atenolol in the Pharmacogenomic Evaluation of Antihypertensive Responses (PEAR) study are included in this study. A targeted LC/MS assay was utilized for the quantitative measurement of 57 ACs in baseline serum. 23 ACs (no or low quantification) and 1 patient (outlier values) were excluded. For the ACs detected in 90% of all patients (n=29), we conducted linear regression for change in glucose (difference between baseline and end of atenolol) with baseline AC level, baseline glucose, age, sex, and BMI as covariates. For ACs detected in at least 50% of the patients (n=5), we modeled change in glucose as described, but with AC included as present/absent in the regression. Patients were 49.7±9.4 years old, 48% female, with mean body mass index of 30.2±5.5 kg/m2, mean blood pressure 145/93 ± 9.4/5.6 mm Hg, mean baseline glucose of 91.4±10.3 mg/dl and mean glucose change of 2.2±9.3 mg/dl. We identified a cluster of long-chain ACs that were associated with glucose change following atenolol treatment. Presence (vs absence) of arachidonoyl-carnitine (C20:4), p=0.0007, as well as increasing concentrations of stearoyl-carnitine (C18), p=0.04 and palmitoyl-carnitine (C:16), p=0.04 was significantly associated with atenolol-induced hyperglycemia. Additionally oleyl-carnitine (C18:1), p=0.05 and linoleoyl-carnitine (C18:2), p=0.07 trended towards significance. Previous reports have identified the association of long-chain ACs with obesity, insulin resistance and diabetes. Additionally, there is evidence from our prior work that the oleic acid, palmitic acid and linoleic acid levels are significantly associated with increased blood glucose after atenolol treatment, and data from the literature demonstrate that arachidonic acid is strongly associated with glucose regulation. Hence, our findings in this RCMRC pilot study, the first to our knowledge, to associate ACs with drug induced hyperglycemia, are biologically plausible and worthy of further investigation and confirmation. Precision medicine and data-driven prescribing is quickly becoming a reality due to revolutionary advances, especially pertaining to “omics platforms”. Application of pharmacometabolomics to classify individuals into subpopulations that differ in their response to a specific treatment is an important enabler of precision medicine. The data from our pilot study suggest that the AC signature in blood serum from patients treated with a β-blocker may yield an informative biomarker that can be leveraged to devise a personalized approach for the treatment of hypertension. Pharmacometabolomics, a tool that identifies signatures related to drug response or adverse effects, can inform the goal of precision medicine.

POSTER 149

Impact of Muscle Insulin and IGF-1 Signaling on Serum and Muscle Metabolite Profiles

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Skeletal muscle insulin resistance is a cardinal feature of the pathogenesis of type 2 diabetes. Insulin resistance is strongly associated with altered mitochondrial function, but whether one causes the other is debated. Altered plasma amino acid (AA) metabolites, including increased branched chain amino acids (BCAAs), occurs in insulin resistant and deficient states and are reported to predict type 2 diabetes in people. Indeed, insulin deficiency in humans causes muscle protein degradation and increased efflux of amino acids into the systemic circulation. However, it remains to be determined whether changes in muscle mitochondrial function or circulating amino acid metabolites occur due to direct impact of reduced insulin action on muscle or secondary to other systemic changes related to the pre-diabetic state. We generated mice with muscle-specific deletion of both insulin receptors (IR) and IGF-1 receptors (IGF1R) using Cre lox recombination. These MIGIRKO mice do not develop diabetes, but do display marked muscle atrophy. To test how loss of insulin/IGF-1 signaling in muscle perturbs amino acid and mitochondrial metabolites, we performed quantitative metabolite profiling on serum and muscle from MIGIRKO mice as well as mice lacking IR or IGF1R alone in muscle, and compared these changes to insulin resistant diet-induced obese (DIO) mice. We correlated changes in metabolites with analyses of mitochondrial functional as well as measures of protein turnover within muscle. Tricarboxylic acid (TCA) cycle metabolite profiling in muscle from MIGIRKO mice showed accumulation of glutamate, fumarate, and citrate; changes that were not present in DIO mice or mice lacking IR or IGF1R alone. These TCA metabolite changes in MIGIRKO muscle agree with observed decreases of mitochondrial respiration in isolated mitochondria using glutamate/malate as substrates, a phenomenon that was not observed with succinate as a substrate. These data demonstrate that loss of IR/IGF1R signaling impairs mitochondrial respiration through complex I leading to accumulation of glutamate and TCA cycle metabolites. Serum amino acid metabolites from of MIGIRKO mice showed minimal changes, without any changes in BCAAs. However, amino acid metabolite profiles from MIGIRKO muscle tissue display increases in BCAAs and many other AA metabolites that are typically altered with insulin deficiency. Elevations in BCAAs also occurred in muscle tissue from DIO mice compared to chow diet controls. Muscle from MIGIRKO also showed marked decreases in histidine metabolites and changes in glutamate and aspartate metabolites, which were associated with increases in markers of protein degradation via autophagy–lysosome. To determine if inhibition of autophagy could change amino acid metabolites in muscle from MIGIRKO or DIO mice, we treated these and control mice with colchicine, a known inhibitor of autophagy in muscle. While autophagy inhibition had minimal effects on metabolites from MIGIRKO muscle, DIO mice showed normalization of muscle BCAA levels with colchicine treatment. Thus, disruption of insulin/IGF-1 signaling in muscle induces mitochondrial dysfunction and alters BCAA/other AA metabolites in part via autophagy-lysosomal degradation.

POSTER 150

Metabolic shift in response to fasting and insulin infusion

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Northern elephant seals show a remarkable plasticity of their metabolic network. They are able to switch between extreme stages of weight gain and prolonged fasting without adverse effects on energy balance. The prolonged fasting of the Northern elephant seal is characterized by reliance on lipid metabolism and conservation of protein. Insulin secretion caused by glucose infusion typically reduces plasma levels of free fatty acids at the early stage of fasting. An atypical elevation of free fatty acids suggests a physiological state that is insulin-resistant like in the adipose tissues of late fasting animals. Therefore, to better assess this insulin-associated shift in substrate metabolism, we compared response of plasma metabolites to insulin infusion in early- and late-fasted Northern elephant seal pups. We demonstrate the response of insulin infusion (65 mU/kg) on 428 circulating plasma metabolites, including free fatty acids, endocannabinoids, and primary carbon metabolites in early and late fasting elephant seals. Metabolomic measurements using three mass spectrometry platforms, LC-MS/MS, GC-MS, and GC-TOF, were acquired at five time points over 2 hours including pre-infusion controls. Statistical analysis, unsupervised clustering, and multivariate modeling identified significantly altered metabolite baseline measurements and time-course excursions (quantified as area under the curve (AUC) values) between early and late fasting animals. Prolonged fasting increased baseline levels of ketones, fatty acids, and endocannabinoids, with average increases of 240%, 82%, and 74%, respectively. Conversely, amino acids and primary carbon metabolites show lowered baseline values in late fast, with average decreases of 51% and 36%. Pathway enrichment analysis of significantly different metabolites identified ketone and branched-chain amino acid metabolism as the leading perturbed pathways. The large cluster of fatty acids show a major decrease in AUC values from early to late fast, with all late-fast AUC values of fatty acids being negative. Clusters of ketone bodies and endocannabinoids follow the same trend. Primary metabolites and amino acids, in contrast, display increased AUC values in late fast, and most late fast time-course excursions are positive. In the early fast the majority of metabolite trajectories return to baseline value within 2 hours; however, metabolite concentrations in the late fast were not able to recover from the insulin perturbation within the monitored timeframe. The insulin-induced increase in fatty acid and ketone body pool size, coupled with the decrease in amino acids and primary carbon metabolites in late-fasting seals points toward a shift towards lipolysis, beta oxidation, ketone metabolism, and decreased protein catabolism. In addition, tight coupling of free fatty acids with the TCA cycle but a delayed response to insulin infusion emphasize the predominant role of fatty acids during prolonged fasting. Primary metabolites, on the other hand, all show increased AUC values in response to insulin in late fast, indicating activation of gluconeogenic precursors. Comprehensive metabolomics combined with metabolic network analysis shows how a mammalian model overcomes an extreme physiological state of prolonged fasting.

POSTER 151

Metabolite Changes Associated with Obesity and Weight Loss

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A chronic imbalance between energy intake and energy expenditure results in excess fat deposition and the development of obesity. Obesity induces changes at the gene expression, protein and metabolite levels in peripheral tissues as well as the central nervous system that drive the development of associated co-morbidities including insulin resistance, diabetes and cardiovascular disease. In these studies we investigated the metabolic changes induced in mice by obesity after chronic high fat diet feeding and after weight loss. We performed both untargeted (complex lipids) and targeted (Endocannabinoids/ oxylipins/ceramide) metabolomic analysis of insulin target tissues from lean, obese and lean mice that were previously obese. Male C57BL6 mice were divided into three groups. One group was fed a high fat (HF) diet (60% calories from fat, Research Diets) for 18 weeks to induce obesity. A lean group was fed a low fat (LF) diet (10% calories from fat, Research diets) for 18

weeks. A third group was fed the HF diet for 9 weeks and then switched (SW) to the LF diet for a further 9 weeks to induce weight loss. At the end of the study mice were sacrificed and tissues (liver, adipose, muscle, hypothalamus and plasma) collected for metabolomic studies. We have identified changes in metabolites that occur in the key insulin target tissues, including adipose, liver, muscle, hypothalamus in the obese state that are reversed upon weight loss. These reversible metabolites are likely associated with insulin resistance in the obese state and insulin sensitivity in the lean state. Furthermore, we have identified metabolites that are induced by obesity that do not reverse upon weight loss. We hypothesize that metabolites that are irreversibly changed in obesity may play a role in driving weight regain and may provide important targets for therapeutic intervention in maintenance of weight loss. Determination of the extent to which weight loss reverses the metabolomic changes induced by obesity

POSTER 152

A graph database atom-resolved implementation of KEGG metabolic pathways

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Metabolomics is a systematic study of the metabolites (small biomolecules) present in a cell, tissue, organism, or community of organisms. Meaningful interpretation of metabolomics datasets requires analysis of these datasets within the context of metabolic networks that create, utilize, and consume metabolites. This is especially true for stable isotope-resolved metabolomics (SIRM) datasets that contain data representing the incorporation of stable isotopes into specific atoms of detected metabolites. To facilitate the interpretation of SIRM datasets, we have created atom resolved metabolic networks using biochemical reaction information from the Kyoto Encyclopedia of Genes and Genomes (KEGG) [1]. Atom-Resolved KEGG or ARK remodels a subset of KEGG as a graph of nodes within a graph database. Nodes in the graph represent everything from atoms, molecules, and enzymes up to genes and organisms, with edges between nodes representing relationships between these entities. The ARK software package synchronizes an SQL database with various relational tables representing entities from KEGG, which are then processed to create atom entries from the KCF fields of compounds (i.e. Mol file-like chemical format). Edges connecting atom nodes across reactions (mappings) are created from the ALIGN sections of KCF fields from reactant pairs (RPairs), with additional mappings created by calculating all possible combinations of molecular symmetry between the reactant-product pairs using an enhanced version of Chemically Aware Substructure Search (CASS) previously developed [2]. Finally, ARK uses the SQL representation of the graph to create an analogous representation in a high-performance neo4j graph database. In this form, simple graph database queries can be used to find paths between atoms in source metabolites and destination metabolites, facilitating pathway-specific interpretation of stable isotope tracing data. Furthermore, these query methods are orders of magnitude faster than traditional SQL queries, allowing systematic analyses of possible isotope tracings between metabolites. References 1.Kanehisa M, Goto S: KEGG: kyoto encyclopedia of genes and genomes. Nucleic acids research 2000, 28(1):27-30. 2.Mitchell JM, Fan TW-M, Lane AN, Moseley HN: Development and in silico evaluation of large-scale metabolite identification methods using functional group detection for metabolomics. Frontiers in genetics 2014, 5:237. We created a graph database implementation of KEGG metabolic networks that are atom-resolved and directly atom-traceable at computationally feasible efficiencies.

POSTER 153

Grinn: A graph database and R package for omics integration

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Typically small molecule metabolites are the downstream products of enzymatic reactions that are mediated by gene-encoded proteins. It is, thus, possible to link genotype to phenotype through metabolomics integration. However current approaches summarize metabolomics analysis in the domain of functional or pathway enrichment, which omits connections among other key molecular components,

e.g. proteins and genes, and as such limits systems level understanding. Grinn (A graph database and R package for omics integration) is a bioinformatics platform that composes of a graph database, an R package and web application. In this version, KEGG is the main resource of metabolic pathways information, which is stored in Neo4j graph database. OpenCPU system is used for the integration of R and JavaScript library to drive the web application. The database is available at http://grinn.genomecenter.UC Davis.edu:7474/browser/ .The online web application is at http://grinn.genomecenter.UC Davis.edu/ocpu/user/kwanich/library/grinn/www/ and the R package for local uses is available from Github (https://github.com/kwanjeeraw/grinn). A platform for omics integration, Grinn, was developed. It is an R application aiming to support metabolomics studies. KEGG metabolic pathways are used to derive connections between the molecular components. Currently, the relationships include metabolite-reaction, reaction-enzyme/protein, reaction-pathway and protein-coding gene associations, which are captured in the database. A graph database is utilized not only to handle the complex relationships among the molecular components, but also to improve speed when querying these highly connected molecules. Two types of networks are generated: a network of connected metabolites and an integrated network of metabolites, proteins, genes and pathways. Network calculation is done through encapsulated R functions. A queried network is presented as an interactive graph and can be exported as a static image or tables for further network analyses and visualization. Grinn broadens metabolomics studies by mapping omics data including genomics, transcriptomics, proteomics and metabolomics for a systems level exploration.

POSTER 154

PredRet: Comprehensive sharing and mapping of RPOSTER LC retention time information

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Increasingly sophisticated and more automated approaches to interpretation of mass spectra have long been the cornerstone of LC-MS based compound identification. Therefore the construction of MS databases have received considerable attention in the metabolomics community. While using fragmentation for compound identification is a powerful tool, it disregards fully half of the information provided by LC-MS. For LC there are currently no coordinated efforts to share and exploit retention time (RT) information. RT information has been neglected in LC because the RT is specific to the chromatographic setup and there are no established RT references. A database of compounds' RTs was therefore constructed and used to predict the RT of compounds in systems where the RTs had not been experimentally determined. RTs of a number of compounds were experimentally determined in two different systems, andused to build monotonically increasing smooth generalized additive models between the RTs in the two systems. Building these models between all chromatographic systems in the database allowed the prediction of the RT for a high number of compounds in systems where they had not been experimentally determined. The prediction tool has been made available as a web application at www.predret.org. The user can upload a spreadsheet with RTs of compounds measured in their system along with molecular identifiers such as PubChem CIDs or InChIs. This data is added to the database, models are calculated and predictions presented to the user. The initial database of RTs for > 2000 compounds was built from several in-house databases containing several hundred compounds each, 9 datasets available on MetaboLights containing a total of 640 compounds and the extensive dataset published by the Metabolic Systems Research Team at RIKEN covering 360 compounds. An easy to use web interface, available at www.predret.org, was built for uploading experimental RTs and downloading RTs as predicted for the user's own system. Building models between all chromatographic systems in the database allowed for prediction of the RT of compounds in systems where they had not been experimentally determined. The number of compounds for which RTs can be predicted and the accuracy of the predictions is dependent on the number of compounds measured in both systems used in the mapping. With the current small database it was possible to predict up to 500 RTs with a median error between 0.02 and 0.25 min depending on the system. The median width of the confidence interval for each prediction currently ranges between 0.1 and 1.7 min. The database can also be used to pinpoint likely erroneous user-reported RTs by comparison with predictions made from the models. We found that the majority of datasets contained entries with either misidentified compounds or incorrectly reported RT. We believe that this tool will greatly help the identification process since compounds that are not

compatible with the observed RT can be disregarded. Confirmatory experiments can then be reserved for compounds that could have the observed RT. This will allow researchers to complete the feature annotation and compound identification process in a faster and more rational manner and thus save time and resources, both monetary and environmental. Community support is required to expand the database and thus dramatically improve the accuracy and coverage of the RT predictions. First system to allow highly accurate mapping and prediction of retention times between any reversed-phase-LC systems.

POSTER 155

Integrating the Chemical Translation Service in automated workflows of MassBank of North America system and other chemical data analysis software

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The Chemical Translation Service 2 (CTS2) is a web application, which aims to fill the gap left by the major Chemical Databases, the 'interconversion of chemical identifiers' Matching hundreds of structures obtained form automated analysis to their chemical names and/or other identifiers can be very straining and time consuming when using only the available solutions. This application can help reduce dramatically the time scientists require to collect the information they need about chemical compounds, to be used in reports, as part of research or even publications. This version of the system has been rewritten from the ground up, maintaining its predecessor's goals, to improve its functionality through the use of newer technologies The CTS2 has been rewritten to add important features like chemical name resolution and a scoring system based on biological importance; using a combination of metrics from Chemspider it gives the user an idea of how strong is a compound linked to biological systems. Also through the use of new technologies like 'RESTful web services' the CTS2 exposes its functionality not only through a web browser but also as an API that can be used by programmers to automate and customize the information gathering or enhance their software by presenting a more complete set of data Our database currently contains over 50 million compounds, which link to over 250 million identifiers and synonyms. This application can convert single or multiple items, between over 200 identifiers among which are the mayor biological and chemical databases HMDB, KEGG, Chemspider, Pubchem, BioCyc, ChEBI, LipidMaps. The conversion times vary depending the complexity and accuracy of the input, having conversions that involve 'Chemical Names' as one of the most complex steps to solve. In the simplest case the conversion of an InChIKey to PubChem CID for example, is roughly under a second using the web interface while the same conversion using the REST service takes about half the time. The conversion times of commonly searched compounds are greatly improved by the presence of caching servers and load balancers that store previously converted values, avoiding the delays of re-creating this information every time. The results of a query using the web interface can be exported as an excel file for later use. Information from Google analytics shows that the system has in average between 200 and 250 users every month and that it's used from all around the world, with the most requests coming from the US followed by Germany, France, UK, India, China to name a few locations. The CTS2 has been integrated already in several systems and applications for example: MassBank of North America, BinBase, RMassBank, CTSgetR. We are currently implementing an automated way of keeping the CTS2 database up to date, reflecting PubChem and Chemspider changes and corrections at the earliest stage possible REST web services. Generation of InChI Codes and InChIKeys from MOL Conversion from InChI Code and InChIKey to MOL

POSTER 156

Massbank of North America: integration and API implementations

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Massbank of North America (MoNA) is a web-based, metadata-centric, auto-curating repository designed for efficient storage and querying of mass spectra and associated structures. MoNA intends to serve as

the framework for a centralized, collaborative database of metabolite mass spectra and metadata. The database currently contains over 180,000 predicted and 40,000 experimental spectra. In order to encourage participation and contributions of mass spectra to the MoNA database, we developed several means by which users may interact with the repository system. We present the current state of the MoNA web application, integration status of MoNA into prevalent processing and analysis software as well as the library implementations of the web API that are currently available in several programming languages. MoNA is a REST-based web application that defines a system of URL "endpoints" used to communicate and interact with the database system. All requests to the system involve navigating to the appropriate URL and, if necessary, submitting a JSON object as defined in the MoNA documentation. While such complex commands are not practical for users to perform, they are ideal for creating a standardized form of communication that may be then be utilized by developers to integrate MoNA with new or existing software. Software must communicate with the following required services for access to the bulk of the available functionality: user authentication and validation, mass spectrum querying and submitting, metadata service, and tagging service. MoNA will primarily be interfaced through the web application (http://mona.fiehnlab.ucdavis.edu), which allows users to browse mass spectra and compounds, perform advanced queries on mass spectral properties available, and upload mass spectra in MSP, MGF, Massbank Record, or MoNA JSON formats. It also provides simple visual representations of many valuable statistics including the distribution of metadata and tags in the database and details about uploaded mass spectral libraries. In addition, users may query MoNA for related mass spectra using implemented spectral similarity algorithms. We have also developed sub-modules to interface MoNA from MS-Dial, MZMine and BinView. From within these applications, a user may login to his or her account, retrieve existing libraries, and upload mass spectra. Features exist to select analyzed mass spectra individually or in bulk, assign compounds and structures, and add metadata (such as instrument, column, collision energy, ion mode, precursor ion and adduct information) and tags to spectra before submission to the server. Additionally, in order to encourage integration into additional tools, API libraries have been written to communicate with the MoNA REST services in Java, C# and R. We also provide full documentation of the MoNA REST endpoints, supporting advanced queries and data retrieval, which may be used in the development of information systems. MoNA allows for querying of dynamic and curated libraries and uploading of mass spectra and libraries from within existing tools.

POSTER 157

PathWhiz: A Web-enabled Pathway Drawing Tool

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PathWhiz (http://smpdb.ca/pathwhiz) is a web server designed to create colourful, visually pleasing, and biologically accurate pathway diagrams that are both machine-readable and interactive. PathWhiz is accessible from almost any place and compatible with essentially any operating system. It also houses a public library of pathways that can be easily viewed and expanded upon by its users. PathWhiz consists of three major components: Pathway Editor, Pathway Viewer and PathWhiz Data Repository for capturing metadata about each pathway and pathway object or process. Pathways are generated and edited using the Pathway Editor while the Pathway Viewer is for visualizing, printing and downloading finished pathways. PathWhiz was built on a Ruby on Rails (version 4.2.0) web framework incorporating a MySQL relational database to manage all of the pathway data. The front-end web client is controlled by Ruby on Rails combined with Backbone.js as the front-end web framework for the editor. PathWhiz's image generation uses the PhantomJS WebKit while custom Java plugins have been developed for BioPAX, SBGN-ML, and SBML generation using the Paxtools, JSBML and LibSBGN libraries, respectively PathWhiz allows users to readily generate biologically complex pathways by using a specially designed drawing palette to quickly render metabolites, proteins, nucleic acids, membranes, subcellular structures,

cells, tissues, and organs. Both metabolic and protein/gene pathways can be constructed quickly and intuitively by combining multiple pathway processes such as reactions, interactions, binding events, and transport activities. PathWhiz's pathway replication and propagation functions allow existing pathways to be used to create new pathways or for existing pathways to be automatically propagated across species. PathWhiz pathways can be saved in BioPAX, SBGN-ML and SBML data exchange formats or they can be saved as PNG, PWML, HTML image map or SVG images that can be viewed offline or explored using PathWhiz's interactive viewer. PathWhiz has been used to generate over 700 pathway diagrams that are being used in a number of popular metabolomics databases PathWhiz is the first fully web-enabled interactive pathway drawing tool, specifically designed to help researchers with pathway/model generation and visualization.

POSTER 158

Recent developments in the MetaboLights database

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Since its official launch in summer 2012, EMBL-EBI has established the MetaboLights database as the most successfully open metabolomics repository internationally. MetaboLights is growing very rapidly and currently host MS and NMR experiments, complete with raw and processed data. MetaboLights is endorsed by publications and fully supports secure private access to restricted or pre-publication datasets. The team is currently working on new features, mainly around integrated workflows and data analysis. With the success of the MetaboLights repository, EMBL-EBI is now focusing efforts into integrating online data analysis. In addition to general enhancements to the current infrastructure, EMBL-EBI is working on a new concept named MetaboLights Labs. Here we will introduce online integrated data analysis tools. The first notable integration is MetaboAnalyst 3.0, kindly provided by David Wishart's lab. The large amount of publicly available primary research data in MetaboLights, an average size of 20Gb per study, is ideal for further analysis and refinement. All public data in MetaboLights is freely downloadable for any purpose, and we continue to advocate open data access. MetaboLights is experiencing a very rapid growth, clearly serving the community in a proactive manner. Currently, MetaboLights has about 150 complete metabolomics experiments with some 50000 samples and 1000 protocols. In addition to this, there are some 16000 reference metabolites with detailed information about the chemistry, pathways, reactions, spectral references and literature. There are about 1600 different organisms reported from studies and reference compounds. MetaboLights is endorsed by journals, like Nature Scientific Data and Metabolomics, and fully supports secure private access to restricted or pre-publication datasets. EMBL-EBI initiated and is a founding member of MetabolomeXchange.org, an open portal for publishing, searching and identifying open access metabolomics datasets. Complete metabolomics experiments, manually curated to a concise and high standard. Online data analysis.

POSTER 159

MASTR-MS Consortium: An international collaboration towards the development and usage of an open-source, standard compliant, LIMS solution for Metabolomics

MASTR-MS is one of the first open-source LIMS solutions specifically designed for metabolomics laboratories that integrates the complete metabolomics workflow. MASTR-MS was originally designed and developed by Metabolomics Australia, in collaboration with partners at the Australian Bioinformatics Facility, Murdoch University. MASTR-MS has three modules: 1. The User Management System handles user accounts, privileges, quotes and client communication. 2. The Sample Management System handles the setting up of projects, designing experiments, defining samples and the creation of sample lists to be used to run samples in the instruments and 3. The Data Management System handles the automatic capture of raw data from the instruments and the systematic storage of processed data and support files related to experiments and projects. MASTR-MS has moved from an advanced development phase to being adopted by members of the Metabolomics community over the last year. In particular, MASTR-MS has now been deployed at multiple nodes of Metabolomics Australia, and is also being used by researchers at the metabolomics facilities at the National U Singapore and EMBL Heidelberg in Germany and the Metalloproteomics facility at the Florey Institute of Neuroscience and Mental Health in Australia.

Researchers at Metabolomics Australia, EMBL-EBI, the U Oxford e-Research Centre and U Birmingham in UK have been collaborating to enhance the functions and features to MASTR-MS, including an experimental detail export and deposition capability to the public repository Metabolights at EMBL-EBI, relying on ISA-Tab data exchange format. We are establishing the MASTR-MS Consortium, comprising of all current users and other interested parties. The consortium will include Metabolomics Australia, EMBL-EBI, UK, the Oxford e-Research Centre at Oxford University, UK, U Birmingham, UK, EMBL Heidelberg, Germany and National U Singapore. This consortium will create a forum in which both users and developers of MASTR-MS can come together to exchange ideas and work at MASTR-MS features, streamline the metabolomics data management pipeline and establish efficient data management practices. We invite metabolomics research groups and facilities to join this initiative to establish a community-wide, community-driven, open source and standard compliant LIMS solution for Metabolomics. Site: https://sites.google.com/site/mastrmsconsortium/ Discussion Group: https://groups.google.com/d/forum/mastrmsconsortium Twitter: @mastrms An international collaboration towards the development and usage of an open-source, standard compliant, LIMS solution for Metabolomics

POSTER 160

Distributed data sharing node for mass spectra repositories in the Metabolomics Node Network

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Recently, the metabolomics community started adopting data sharing practices to effectively utilize and manage large MS datasets. Databases are gradually opening public access to their data and analysis tools, and supporting service integration using APIs. However, managing local servers large datasets is complex, tedious, and expensive for most research groups. Excessive duplication of spectra data also wastes storage and network resources. In light of this, the Metabolomics Node Network (mnn) provides a data node for storing mass spectra datasets. It is designed as a web-based repository management system deployed in a self-contained web server. mnn aims to provide a long-term strategy for addressing redundancy of spectra data. This project's target users are MS research groups who want to manage experimental data in a personal repository, and easily incorporate it into their workflow. Various metabolomics data services, as well as collaboration and data integration systems for general use, were studied for feature sets and software architecture concepts suitable for the system's target users. Moreover, popular web standards and technologies similar to existing MS services were incorporated to ease integration efforts. The data node is developed using Java and Scala, while its front-end was built on AngularJS. The data exchange format used is the JSON variant of the Hypertext Application Language. Institutions can install one or more data nodes where each data node hosts multiple

repositories, all of which are publicly accessible. Repositories are be created and managed by individuals, groups, or made open to the public. A repository can hold MS data in various file formats, as well as documentation files in text format.A data node provides common services such as user management, local search, file format conversion, and resource version tracking. Applications and services can integrate with repositories using its REST API. Data nodes are also made accessible to web crawlers. To save on disk and network resources, repositories are stored in locally and not copied into other locations. Instead, a network-wide registry of repository names and locations is maintained. Similarly, mass spectra is stored in local data nodes, however the network's main crawler is used to identify spectra that can be merged to form a web index.Currently, the system is being tested in a private small-scale environment. Data nodes were deployed across several virtual servers, each hosting repositories comprised of different datasets from the MassBank database. Efficient distributed data management of large metabolomics datasets for long-term growth of the web of MS data

POSTER 161

Data standards and global exchange of data in metabolomics

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Reproducibly of the metabolomics experiments as well as transfer and sharing of experimental results between research groups have become of paramount importance as the filed is maturing. This is even more crucial for publishers to make data set available for reviewers and publically funded research to make the data accessible. Many exciting initial findings are based on pilot studies with small sample sizes, and in-order to be translated into wider applications such studies need to be replicated. Efficient replication depends on making study protocols, data and metadata openly available. However, merely sharing raw data is not as useful without addressing the additional challenges of standardized and open formats, clear and sufficient descriptions of study protocols, and the use agreed standards. In this contribution we outline our effort in COSMOS (COordination Of Standards In MetabOlomicS) for global data standards and data sharing projects. In addition the role of open access data exchange format in relation to metabolomics repositories, particularly MetaboLights the first global-scale, open access repository for metabolomics studies and MetabolomeXchange.org where publically availed metabolomics studies are broadcast and announced. Here we describe our concepts and efforts in engagement with the metabolomics community, academics and industry, journal publishers, software and hardware vendors, as well as those interested in standardisation worldwide to addressing missing metabolomics ontologies, complex-metadata capturing and XML based open source data exchange format. In addition, COSMOS has developed the missing XML exchange formats for NMR spectroscopy nmrML and working toward capturing identification and qualification NMR data in a nmrTab format. These developments take place on www.nmrml.org and https://github.com/nmrML/. Finally, we would like to report on our effort in

capturing and reporting fluxomics and metabolomics based imaging datasets and experiments. With semantic web technology in mind, these standards will pave the way for metabolomics data to be part of the world of linked (and open) data while MetaboLights will serve as a common publication hub and make it possible to connect different resources while keeping the data interoperable. keywords: metabolomics data exchanged standards, MSI, PSI, RDF, open formats, XML and linked data,

POSTER 162

Accurate Mass for Improved Metabolite Identification via High-Resolution GC/MS

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Gas chromatography/mass spectrometry (GC/MS) is a premiere analytical tool for qualitative and quantitative analysis of volatile organic compounds and is key technology for the emergent field of metabolomics. Harnessing this potential, however, has been challenging for numerous reasons: pseudo-molecular ion information is often not present, co-elution of multiple compounds confounds identification, and the largest spectral libraries exclusively contain low resolution spectra. All of these factors have limited the impact of GC/MS for metabolomic application. Here we describe a novel combinatorial informatics approach, High-Resolution Filtering (HRF), which exploits the accurate mass capabilities of newly introduced GC/MS platforms. Our HRF method augments traditional database searching by in silico fragment matching for greatly increased confidence in metabolite identifications. Please consider for travel awards. The underlying assumption behind the HRF strategy is that every fragment derived from a particular precursor must contain a subset of atoms of that precursor. More directly, we assert that every peak in a pure high-resolution GC/MS spectrum can be annotated using a combination of atoms from the true precursor. Given a high-resolution GC/MS spectrum and a putative identification, all non-repeating combinations of atoms from the assigned chemical formula are generated and then matched to peaks according to exact mass. While some combinations represent chemically impossible formulas, this list inherently contains all formulas for fragments which could possibly be observed. Here we demonstrate that the current implementation is viable, facilitating straightforward discrimination between correct and false assignments. We tested our approach using a dataset of high-resolution GC-Orbitrap (a GC interface with EI/CI source on a Q Exactive instrument platform, Thermo Fisher Scientific) spectra collected from 105 pure reference standards covering many classes of small molecules. Individual spectra were extracted from raw data files using an in-house deconvolution algorithm designed to group together those fragments stemming from a singular precursor. Spectral quality was ensured by requiring that extracted spectra closely resemble their corresponding unit resolution reference spectrum in the NIST 12 EI Database. Using our HRF strategy we attempted to annotate the maximal amount of ion current in each spectrum using only non-repeating combinations of atoms from a selected parent formula. Considering only true identifications, we achieve 99.7% annotation of all observed signal. To fully characterize the specificity of the approach we calculated an HRF score for each spectrum in the dataset using 60,560 unique chemical formulas from the NIST 12 EI Database. We find that on average ~86.9% of considered formulas will return a HRF score < 90 and that only ~3.6% of considered formulas will return a score greater than or equal to the median calculated HRF score (99.7) indicating that the method is strongly specific towards correct precursor assignments. Finally, we tested algorithmic performance when identifying standards spiked into human urine at variable concentrations (10ng/uL to ~78 pg/uL). We note that spectral match scores tend to decrease with diminishing analyte abundance. However, we also find that the calculated HRF metric remains high even as concentration declines suggesting that mass accuracy is highly conserved and that the HRF metric is robust in times of reduced S/N. Overall, the HRF approach provides a direct path to exploit existing low resolution EI mass spectral databases for use with state-of-the-art high accuracy GC/MS systems. We report a novel combinatorial approach for leveraging accurate mass to improve metabolite identification specificity via GC/MS.

POSTER 163

Creation of High Quality Metabolite Libraries for Fast Metabolomics Screening and Identification

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The study of endogenous metabolites has brought new possibilities in the field of metabolomics, particularly for life sciences research. Compared to genomics and proteomics, identification of endogenous metabolites continues to pose limitations and challenges to many researchers. This is due to the absence of a reliable screening library to provide accurate information and high quality data needed for metabolic profiling studies. Online databases available for metabolite identification often provide numerous candidates that require further analysis to remove redundancy. To overcome the obstacles in identification, we present a compound spectral library of endogenous metabolites. This library contains a repository of accurate masses, retention times and MS2 spectra information, which can be acceptance criteria used to improve the confidence in metabolite identification. 300 commercially available metabolite standards were prepared in mixtures of 25 compounds per sample batch (concentration 0.5mg/ml). These standards mixtures were analyzed on a Thermo Scientific Q Exactive mass spectrometer coupled to an Ultimate 3000 UHPLC system. Chromatographic separation was on a Thermo Scientific Hypersil Gold column (C18, 2.1 x 150mm, 1.9um), 15 minutes LC gradient at 450ul/min flow rate. Two acquisition modes were used: A full MS scan at 35,000 resolution with pos/neg switching and another used full MS at 35,000 resolution followed by data dependent MS2 at 17,500 resolution in pos/neg polarities. All data were processed using the Thermo Scientific TraceFinder 3.2 software. The compound database, MS2 spectra library and screening methods were created with the TraceFinder software. A preliminary list of 4500 endogenous non-lipid metabolites was compiled, which included the compound name, chemical formula, monoisotopic mass and CAS ID information for each metabolite. The list formed the basis of the compounds to be analyzed for the creation of the metabolite library. In a pilot study, 300 commercially available metabolites were chosen from the 4500 metabolite list. Their full MS scan data were acquired to obtain the retention time (RT) information. RT is one of the useful identification criteria for metabolite ID when a standardized chromatographic method is used. Subsequently, the polarity switching method with full MS and data dependent MS2, with multiple collision energies of 10, 30 and 45 was carried out for a subset of 60 metabolites. The purpose was to generate the MS2 spectra required for the creation of the high quality spectral library. A total of 6 MS2 spectra with the respective collision energies of 10, 30 and 45 in positive and negative polarities were obtained for each compound. These MS2 spectra files were submitted to the Library Manager database creation tool in TraceFinder software. A library containing over 300 high quality MS2 spectra for these 60 metabolites was created to be part of the screening solution for the metabolomics workflow. To test the metabolite identification performance of the library, a ZDF rat plasma dataset was processed to identify the possible endogenous metabolites present based on m/z, RT and MS2 spectra. 88 metabolites were identified based on accurate m/z, of which 29 were matched based on RT and 5 metabolites had the MS2 spectra matched exactly. Generated in a few seconds, the results demonstrated that the use of multiple screening features narrows the identity of the endogenous metabolites in the ZDF rat plasma considerably and enables confident identification of endogenous metabolites. A metabolomics library integrated with commercial software enables fast and confident metabolite screening with the use of multiple search criteria.

POSTER 164

Expanded High-Resolution MS/MS Human Metabolite Spectral Library for Clinical Metabolomics

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Human metabolomics as a key approach to discover biomarkers holds the potential to be used for routine clinical test for improving patient outcomes and diagnostics. In non-targeted metabolomic studies,

typically many unknown mass spectral features are profiled. We established a high-resolution MS/MS spectral library containing about 800 human metabolites to facilitate the identification of unknowns. To further improve the confidence of unknown identification, especially for isobaric or isomeric compounds, normalized retention time data was added to this library. This improved the robustness for compound identification, which will be demonstrated here using human urine and serum samples. 1. Standard mixtures were preparedusing endogenous human metabolites obtained from the Human Metabolite Database (HMDB). 2. Each standard mixture was spiked with a pre-prepared retention-time standard mixture that was used for retention time normalization and quality control. 3. Retention times were measured in triplicate using a 20-minute linear gradient in positive and negative modes. A Dionex Ultimate 3000 UHPLC with a RSLC 120 C18 column (Thermo Fisher) and a high-resolution impact HD Q-TOF mass spectrometer (Bruker Daltonics) was used for separation and detection. 4. Data were processed in TargetAnalysis (Bruker Daltonics) for automatic mass calibration and retention time extraction. The previously established high-resolution HMDB MS/MS spectral library provides accurate and quick identification of human metabolites. Adding retention-time data improves identification confidence, especially for low intensity, isobaric or isomeric compounds. Prior to data acquisition, the UHPLC gradient profile was recorded using 0.1% acetone in water and UV detection to ensure the quality of the retention time data, and monitor instrument performance. A simple 20-minute linear gradient was applied for its simplicity and ease of retention-time normalization. The gradient reproducibility was assessed using a mixture of evenly spaced retention-time standards. The retention time deviations for the measured compounds were within 0.21-1.29 seconds (n = 5). Before injection, the HMDB standards were separated in groups by chemical classes to avoid any unexpected complex-formation or reactions. Non-targeted LC-MS/MS analyses were performed for both urine and serum samples by using the established LC gradient. The acquired data were automatically mined using a feature extraction algorithm. These features containing related MS and MS/MS spectra were queried in the spectral database. Matching MS/MS spectra and retention time information enable rapid identification of compounds belonging to different compound classes in both matrices. By analyzing the acquired data, possible matrix dependent retention-time shifts were evaluated and appropriate tolerances were assigned for matching compounds from different biological matrices. We aim to develop and establish a standard operation procedure (SOP) and extend the power for acquiring, normalizing and then searching the HMDB spectral library, to generate accurate identification based on MS/MS and retention-time data applicable to different samples and instruments. Accurate mass and retention-time library of endogenous human metabolites was developed enabling quick metabolite identification in clinical metabolomics research.

POSTER 165

Flavonoid Search: Enhanced annotation of flavonoids through construction of empirical dissociation rules and a predicted mass fragment database

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Flavonoids are a major class of compounds that are targeted by metabolomics studies because of their biological and chemical functionalities; however, high-throughput annotation of each compound remains challenging. Many candidates are found in compound databases for many isomers in this class, even when an accurate mass detected using a high-resolution mass spectrometer (MS) is queried. Multi-stage mass spectra (MSn) can elucidate their chemical structure, though only a limited number of flavonoids are available in MSn spectra databases. However, abundant knowledge about the molecular dissociations of each flavonoid is available in the literature. Therefore, in some cases, expert researchers can predict flavonoid structures from their MSn spectra. As a replacement for the expertise required for one-by-one structural elucidation, we constructed a database of putative MS fragments by applying empirical dissociation rules to known flavonoids. A hybrid system consisting of an ion trap and a Fourier transform ion-cyclotron MS was used for constructing these rules because >MS3 was required to dissociate flavonoid-glycosides using a soft ionization method. We analyzed 132 authentic flavonoids, attributed their MSn fragments to their substructures, and constructed dissociation rules. These rules were then

applied to all flavonoids, and key fragments that could characterize various aglycones were predicted and stored in the database. We developed a tool to search the database using a measured spectrum, and its accuracy was compared with other tools. An enhancement in the predictability of flavonoids, compared to other tools that are used for general metabolites, was obtained with our approach, Flavonoid Search, which uses empirical dissociation rules that are specifically constructed for the flavonoid class. In the construction of these rules, we reclassified the aglycones into 84 sub-classes based on structures related to molecular dissociations in MSn analysis. Applying these empirical rules, the known flavonoids were characterized using 57 of neutral loss, 11 of A-ring, and 13 of B-ring derived fragments. As a result, key fragments were predicted for 4311 out of the 6868 known flavonoids. Our approach resulted in much better predictions than other structure prediction tools using MS spectra, e.g., MetFrag, CFM-ID, and FingerID. These results suggested that using empirical dissociation rules, which are constructed from structural characterizations for a specific compound class, could expand the search space of MS spectra, and enhance annotation. We will report the details of our approach in this POSTER presentation. The annotation of flavonoids was improved using a MS fragment database predicted by empirical dissociation rules.

POSTER 166

Lipid-Pro: A Bioinformatics Tool towards Lipid Identification

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Mass spectra of lipid species in complex biological samples are a challenge to interpret since the lipid composition is specific to organism, tissue and cell. Additionally, thousands of lipid species are present in a biological sample and the number of lipid classes and the isomer are high and diverse. For their accurate identification different measured physicochemical properties, such as accurate mass, fragmentation behavior and a polarity, have to be considered in the pre-processed data. Here, we present our recently proposed bioinformatics tool i.e. Lipid-Pro, a computational lipid identification solution for untargeted lipidomics on data-independent acquisition tandem mass spectrometry platforms. It performs lipidome analysis by analyzing LC-MS/MS data, where defined lipid species are identified in the pre-processed data (lipid profiling). Lipid-Pro is a desktop application and its graphical interface is very simple to install and use. It consists of five main modules: Building Block, Precursor Candidates, Fragment Candidates, Mass Analyzer and Lipid Identifier. Using these modules, user can build up a database by defining building blocks, composing lipid molecular ions and their characteristic fragments and match that to the acquired m/z-values of molecular ions and fragments to identify lipid species. Moreover, it also provide comprehensive data management, sharing and integration features. Lipid-Pro is a well-planned scientific solution, implementing the Butterfly paradigm (Ahmed et al., 2014, PMID: 25383181). Started with the initialization of the finalized scientific solution's requirements, then modeled and mocked using Human Computer Interaction (HCI) guidelines. Lipid-Pro has been recently published at one of the leading Bioinformatics (by Oxford University Press) journals of the world, and to have complete application details (including detailed methodology, workflow, implementation, database, usage and results) please consult Ahmed et al., (manuscript and supplementary material, PMID: 25433698). Lipid-Pro is programmed in C# programming language, using Microsoft Dot Net Framework and only compatible to the Microsoft Windows Operating Systems (preferred OS version: 7). Lipid-Pro is a freely available tool for non-commercial, academic and scientific research, accessible at the following web link: (http://www.neurogenetics.biozentrum.uni-wuerzburg.de/en/project/services/lipidpro/). Recent available version of Lipid-Pro has been successfully tested and validated at the Metabolomics Core Unit at U Wuerzburg.

POSTER 167

Improved untargeted metabolomics with MAGMa through brute-force parameter optimization on the Metlin dataset

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Over the recent years, mass spectrometry based untargeted metabolomics (global metabolome analysis) has made significant leaps forward. The CASMI contest has been created to follow up and stimulate the research on computational untargeted metabolomics assignment methods. Now in its third edition, CASMI has demonstrated there are two major players in the field, MAGMa and CFM-ID. Considering their different underlying principles and complementary behavior with respect to correct assignments, efforts to further invigorate this methodology's capabilities should be divided over both tools. As regards MAGMa, it calculates a matching score between in silico generated molecule fragments and MSn peaks using a formula which involves several parameters. We found the parameter values fixed by the original authors leave room for optimization. The Metlin database contains over 240000 metabolites for most of which a molecular structure can be obtained or generated. For more than 12000 of these metabolites, high resolution MS/MS spectra are also provided. Hence, Metlin forms an ideal dataset for spectrum-metabolite matching evaluation. One by one, these MS/MS spectra are given as input to MAGMa, who is allowed to search in the 240000+ metabolite containing database. The average correct match rank then constitutes a metric for assignment quality. We allowed three parameters to vary relative to the single bond break cost within certain boundaries: double bond break cost, aromatic bond break cost and unexplained MSn peak cost in search for the maximum average correct match rank in this optimization space. So far, we have evaluated almost 1000 different parameter settings. The parameters for single, double, triple, aromatic bond break cost and unexplained MSn peak cost in the publicly available version of MAGMa are set to (1.0, 2.0, 3.0, 3.0 and 10.0) respectively. However, our calculations indicate that when pursuing maximum average correct match rank, these parameter values converge towards the value set (1.0, 3.0, 3.0, 2.5, 7.0). The MS/MS spectra available in Metlin are recorded either in positive of negative electrospray ionization mode using four different collision energies (0, 10, 20 and 40V). From these data we generated four distinct data sets: POSITIVE_ALL, POSITIVE_SELECT, NEGATIVE_ALL and NEGATIVE_SELECT. “all” implies that the spectra at all four collision energies were merged into one spectrum while “select” refers to the single lowest collision energy spectrum for which at least 75% of the molecule is fragmented. These datasets contain (generated) spectra for 10463, 10374, 3699 and 3560 metabolites respectively. Using the original parameter set, MAGMa ranks the correct metabolite first in 5874, 6174, 2278 and 2291 cases. Applying the optimized parameter set, these numbers increase to 5973, 6335, 2321 and 2327. These values correspond to a 1.7%, 2.6%, 1.9% and 1.6% performance improvement. In fact, the actual correct assignment values are higher when wider mz windows are used, however, for our optimization purposes this inconveniently slowed down the calculations. The parameter optimization step forms the first obligatory step in a three-stage improvement procedure that we envision for MAGMa in our goal to bring it to excellence level. The second step will involve MS/MS spectral comparisons among the top selected metabolite candidates. In the final step we aim to combine several tools using learning to rank technology. Currently, we are starting to include the improved MAGMa in our pipeline towards anti-angiogenic therapies. MAGMa's new parameters further establishes untargeted metabolomics as a formidable technology to identify novel metabolic mechanisms in health and disease.

POSTER 168

Biotransformation as a determinant context to explore the exposome.

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Metabolomics and exposome insights mutually illuminate the role of life-style practices towards integrated biosystems models of health and disease. Metabolomics opens an avenue for integration of metabolic-

based life-style consequences into complex biosystems models. Traditional research is hypothesis-driven. Physio-biochemical consequences of life-style practices mostly affects several targets, metabolic pathways and functions, resulting in a magnitude of effects often much lower than from hypotheses-based experimentation. Investigating life-style practice through induced perturbed conditions (e.g. challenge tests) is anticipated to better insights in low magnitude effects often characterizing life-style practices. Here we present the outcomes of a designed metabolomics study on induced acute alcohol consumption, providing insights on the consequences of a potential life-style addiction and the allostasis response of biotransformation to the exposome. We report on a designed longitudinal intervention study (duration: 4 hours) on induced acute alcohol consumption (1.5 mg Vodka per Kg body mass, using 500 ml. Lemon flavored water with benzoic acid preservative as vehicle) by 24 experimental subjects (healthy males, age 20 to 24 years), using a metabolomics approach. The measurement design generated 1176 samples (including 408 QC samples for estimating batch effects) to be analyzed, as well as 288 blanks for demarcation purposes. Data extraction and quantification (relative concentration/mmol creatinine (Cr)) was done through AMDIS and MET-IDEA. Principal component analysis (PCA) for unsupervised pattern recognition and a partial least square discriminant analysis (PLS-DA) as a supervised method were used for dimensional reduction and identification of important variables. A total of 176 features were detected in the QC samples used, including 2 internal standards and 3 features that could not be identified. The Human Metabolome Database (www.hmdb.ca) was used as the reference for the biological description of each feature and used as attribute for their classification. Information on features not in the HMDB was obtained from stablished chemical databases or from the literature, and failure with this resulted in classification of 6 features as “no annotation” and 21 from non-biological origin. The classified metabolites were accordingly: 81 human metabolites; 12 human biotransformation products; 9 from gut microbiotica; 23 from diet and 18 artifacts, mainly formed due to derivatization. Several biotransformation products were glycine conjugates of exogenous substances or due to the alcohol challenge test, indicating an allostasis response towards restoring/maintaining homeostasis, e.g.: (1) Hippuric acid contributed significantly ($p = 0.0000$) to the PCA- and PLS-DA-based differentiation within one hour following alcohol consumption ($\Delta t=0;1$ hour; 1278 µmole/mmol Cr; VIP=13.70; Fold change (FC) = 3.28). This could be expected as hippuric acid is a Phase II conjugation product of benzoic acid (catalyzed by glycine N-acyltransferase (GLYAT, E.C. 2.3.1.13), a major constituent of the vehicle used. Results on three other biotransformation products likewise indicate the central role of GLYAT in biotransformation processes: (2) Tiglylglycine ($\Delta t=0;3$ hours = 4,15 µmole/mmol Cr; VIP 1,72; FC = 4.18) suggests detoxification of increase tiglic acid due to alcohol-induced NAD+-depletion; (3) 2-hydroxyhippuric acid indicates detoxification of a metabolite from the gut microbiotica ($\Delta t=0;1$ hours = 0,24 µmole/mmol Cr; VIP 0.14; FC = 2,95); (4) cinnamoylglycine ($\Delta t=0;4$ hours = 1,81 µmole/mmol Cr; VIP 1,72; FC = 4.18) apparently is derived from cinnamic acid, a very minor flavor ingredient in some alcoholic beverages. Finally, some clear individual variation in biotransformation ability / response between the experimental subjects occurred. Biotransformation should be a determinant in exploration of the exposome and in individual-based criteria for life-style research, assessment and management.

POSTER 169

Assessing Cryptosporidium parvum viability in water using an untargeted metabolic profiling approach

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An outbreak of Cryptosporidium in a water catchment poses significant health risks to the general public. The need to identify and characterise Cryptosporidium viability and infectivity remains a high priority, with regulators demanding regular monitoring. One potential approach is to assess Cryptosporidium viability using metabolomic techniques. Cryptosporidium oocysts have been found to contain a finite carbohydrate energy reserve (amylopectin) which is used to initiate infection within the host. Furthermore, this energy reserve is consumed in direct response to an increase in ambient environmental temperatures and inactivation is a function of increased metabolic activity. This paper demonstrates the application metabolomics for the assessment of Cryptosporidium viability in a number of different waters. Viable and irradiated Cryptosporidium oocysts were sourced from a commercial supplier and inoculated into four

different potable and recreational water sources, representative of pristine to moderately polluted samples. The inoculated samples were exposed to three different temperature treatments (ranging 15 - 37°C) for a period of five days prior to being lysed. The samples were then sonicated and centrifuged prior to removing the supernatant and drying under vacuum. The dried samples were derivatised by 2% methoxyamine-HCl and N,O-Bis(trimethylsilyl)trifluoroacetamide (BSTFA) containing 1% Trimethylchlorosilane (TMCS) using a microwave instrument and analysed by GC–MS. Data acquisition and processing were performed using MSD Chemstation. Metabolites were identified using a mixed alkane standard and Kovats retention indices. Analytical conditions were monitored and optimized accordingly, in order to acquire method validation data. Relative standard deviations (RSDs) were calculated and found to be within acceptable limits for each corresponding internal standard (stearic acid-1-13C (3.89%) and adonitol (5.62%)). Chemometric analysis of the data was undertaken using SIMCA 13 (Umetrics, Sweden). The PCA plot using the GC-MS data was observed to discriminate samples. All the controls, inoculated viable and inoculated irradiated samples occupied different hemispheres of the scatter plot which were indicative of the metabolites that are found within the sample matrix and relating to Cryptosporidium – irrespective of the water type analysed. In total, 49 significant metabolites were observed (p 100). The significant peak features identified included a mixture of aromatic and non-aromatic amino acids, carbohydrates, fatty acids, and alcohols. Organic acids were also identified, but they were not classified as statistically significant features. Qualitative assessment of these peaks indicated that the viable samples comprised metabolites related to polysaccharide degradation pathways, while the irradiated samples comprised metabolites related to lipid degradation. Metabolomic profiling was used to rapidly identify and characterise Cryptosporidium parvum oocyst viability in a range of different water types.

POSTER 170

Mass spectrometric-based metabolomics for determining biomarkers of exposure during fasting in field-based chemical exposure studies

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Fathead minnows are routinely used in field studies to determine biomarkers of exposure to environmental contaminants. These include complex mixtures of contaminants eluting from wastewater treatment plants into the nation's rivers, lakes and other waterways. However, these field-deployed fish are often not fed for the duration of the studies, relying on local sources of food for nutrients, even including wastewater effluents. Complications can arise in these studies, such as unexpected weight gain in fish located closest to the effluent sources, while fish from other deployment locations lose weight, apparently from fasting. Biomarkers of exposure to fasting are extremely difficult to distinguish from those arising from environmental contaminants. Four classes of 108 total fathead minnows are fed brine shrimp at systematically varying rates for four days. Mucus and plasma samples are collected for all fish and are analyzed using a Q-Exactive mass spectrometer coupled to liquid chromatography. Raw data files are converted for preprocessing with the XCMS and CAMERA packages in R. Univariate analysis using ANOVA in R determines the features that vary significantly between classes. Putative metabolite identification is performed with the MetaboSearch program searching the HMDB, LipidMaps, and MMCD databases. Metabolites are confirmed with MS/MS spectral matching to these and the METLIN databases. Multivariate analysis using SIMCA and biological pathway analysis confirm the effects of fasting on the fish metabolome. While the data analysis is ongoing, so far XCMS has identified 2120 potential biochemical features, 852 in positive ionization mode and 1268 in negative mode. CAMERA annotates 1261 of the identified features as isotopic peaks, adducts, or both. About half of the initially detected features have been putatively annotated by searching against the HMDB, LipidMaps, and MMCD databases. Initial multivariate analysis of the plasma dataset with PCA show a significant sex response among the population. Moreover, PLS-DA analysis of the male population indicates that there is a significant change between the not fed class and fully fed class, with intermediate responses for the classes fed at half and quarter rations, relative to the fully fed group. The dataset composed of the female population suffered from significant sample loss due to difficulty in extracting blood from the smaller sex, and as such no fasting response is observed from the plasma dataset. The mucus dataset is

currently undergoing analysis, and we anticipate seeing a similar fasting response among the population. Our goal is to identify those metabolites which vary significantly due to the fasting response yet are similar between the mucus and plasma datasets, in order to determine if mucus is a good surrogate for blood for future field-based metabolomics studies. Mucus as a surrogate for blood in field-based metabolomics studies; MS based metabolomics for environmental exposure studies.

POSTER 171

Frog skin secretome: What does metabolomics reveal?

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The non-keratinized skins of frogs are permeable to water which proximate the frog's internal milieu and the external environment. Frogs sense changes in the environment through its skin and respond appropriately to ensure homeostasis and survival. Frog skin secretions have been a target for chemical prospecting since the seventies, due to its antimicrobial characteristics, amongst others. Despite this, the small molecules in the frog skin secretome remain uncharacterized for many frogs. Small molecule characterization of secretions from different species could give valuable information such as compound functionality, similarities in species or environment responses. The generation of reference profiles could also aid in the screening of bio-active compounds with potential pharmaceutical application, when frogs are subjected to various stressors or pathogens. The secretome of various species frogs were collected through electro-stimulation and kept at -80 ˚C till analysis. Water was added to samples to get secretions in suspension. The protein rich sample were treated with three volumes ice-cold acetonitrile to precipitate proteins. The samples were vortexed and centrifuged to separate the protein pellet from the supernatant. The supernatant were collected and dried under nitrogen. Water and acetonitrile (50:50) were added to the dried samples according to the starting secretion volume. Samples were analyzed with LC-IM-MS (Synapt from Waters) using a C18 column in positive and negative ionization mode. The data were processed with Progenesis QI and compounds annotated using several accurate mass databasis such as Metlin, HMDB, CheBI, LipidMaps, etc. Low energy data were used to perform statistical analysis and illustrate similarities and differences between the species. The high energy data were used to confirm the identities of several compounds with spectral matching and help elucidate potential structures of novel compounds not annotated by any of the databases. Multivariate analysis was performed to visualize the natural grouping of the species and show similarities in secreted compounds. Univariate analysis was used to identify compounds markedly different between them. Preliminary results show marked differences between the species which suggest possible differences in their responses to the environment, albeit when we assume uniformity in the chemical environmental around them. Furthermore, most of the compounds that were found in the collection of frogs were annotated as lipids and also have lipid-like behavior (eluting late from the C18 column). While the identity of many of these compounds is still uncertain and in the preliminary phase, these characterizing results are beginning to open doors in our understanding of the frogs skin secretome. Untargeted LC-IM-MS analysis of various frog secretions and compound identification using MSe and mobility data with Progensis QI.

POSTER 172

Untargeted Metabolomics as Profiling Tool for Nanotoxicity of Copper Oxide Nanoparticles in A549 Lung Epithelial Cells

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Copper oxide nanoparticles (CuO NPs) are widely used and have various applications, for example in cosmetics, microelectronics and as catalysis. The versatile and expansive use of CuO NPs lead to major concerns about the impact they may have on human health. One possible route of exposure is via the lung, therefore a human-alveolar-basal-epithelial cell line (A549) was used as model system for risk assessment. Toxicity profiling was performed using an untargeted metabolomics approach for screening the impact of CuO NP exposure (10 µg/mL) in a time-dependent manner (0, 1, 3, 6, 12, 24 h). Furthermore we aimed to establish and apply an automated data evaluation pipeline using open source tools such as OpenMS and KNIME. Two different approaches were chosen for toxicity profiling, namely reversed-phase and hydrophilic interaction HPLC-ESI-MS. Both measurements were performed on an Orbitrap mass spectrometer in positive and negative ionization mode. The chromatographic separation was done with an UHPLC system employing a polar endcapped C18 phase (Hypersil Gold aQ C18, 100 x 2.1 mm, 1.9 µm) and a HILIC phase (Nucleodur HILIC, 150 x 2.0 mm, 1.8 µm). Bioinformatic workflows using the open source software packages OpenMS and KNIME were applied for data processing and evaluation to determine differentially expressed features. These were subsequently either annotated by comparing accurate masses with the human metabolite database (HMDB) and MS^2 fragmentation patterns with Metlin or by using a reference substance for identification. The bioinformatics software tools OpenMS and KNIME were suitable for processing untargeted metabolomics HPLC-MS data, as they allowed fully automated raw data processing, filtering according to different quality criteria, and the opportunity to perform statistical analysis. The application of this data evaluation pipeline for toxicity profiling of CuO NPs resulted in individual datasets for each method, containing several hundred authentic features that passed the filtering criteria, which was subsequently used to determine differentially regulated features/metabolites by applying Linear Model for Microarray Assays (LIMMA) and Principal Component Analysis (PCA). PCA scores plots revealed differences between controls (medium only treated cells) and treated samples after 6 hours, which were even more pronounced for the longer treatment periods. For each time point, significantly regulated features (Poster value < 0.05) were determined using LIMMA. This univariate statistical approach was in line with PCA and confirmed that the number of differentially expressed features was rising from 6 hours on. These features were annotated by accurate mass and fragment spectra search, which led to 30 and 25 regulated metabolites for HILIC and RP separation, respectively. If possible, the identity was confirmed with use of reference substances. Different classes of metabolites were identified, amongst them: a general increase in amino acids, albeit with several carnitine species having a contrasting trend; and elevated levels of citrulline and cysteineglutahione disulfide and a reduced concentration of glutathione and hypotaurine, indicating oxidative stress. Furthermore, increased levels of glycerophosphocholine and 5'-methylthioadenosine upon treatment were associated with apoptotic events. These findings suggest that treating A549 cells with a concentration of 10 µg/mL CuO NPs lead to oxidative stress as well as to apoptosis after 6 hours of incubation. This hypothesis was proven in further cell assays measuring Heme oxygenase 1 (HO-1) gene expression, for oxidative stress, and activation of a caspase cascade for apoptosis. Metabolomics used for NP toxicity profiling in a time dependent manner and the application of novel strategies for data evaluation.

POSTER 173

Exposome-Wide Association Study (EWAS) using GC-QTOF and targeted follow up for persistent organic pollutants (POPs) in plasma using GC-MS/MS

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Sophisticated tools were developed to sequence the human genome and these have been harmonized across genomics research. Although refined procedures in mass spectrometry exist to simultaneously measure many chemical exposures, there are few applications and little harmonization. In this study, non-hypothesis driven EWAS was performed using GC-QTOF for fifty-one un-paired case / control plasma samples. Statistical analysis was performed to elicit putative chemical entities and chemical features in each population. A knowledge-driven follow up study using targeted GC-MS/MS and secondary statistical analysis was used to confirm and validate the findings. This GC-MS EWAS / targeted model can be used as a basis for method harmonization in conjunction with other technologies such as LC-MS, ICP-MS and NMR. An Agilent 7890B GC and 7200A QTOF was used for EWAS. The injection port was 280oC. The

column was a HP-5MSUI (P/N: 19091S-433UI). The oven program stepped from 80°C to 325°C. The transfer line was 300°C and source was set to 350°C. The MS was run at 5 Hz acquisition rate over 50 – 650 m/z. An Agilent 7890B GC and 7010 triple quadrupole mass spectrometer was used for the targeted follow up study. The GC conditions were as above. The GC-MS/MS was run in EI MRM mode. The collision gas was nitrogen. Targeted MRM was performed for 66 POPs representing six chemical classes. Mass Profiler Professional (MPP) software identified significant features in the non-targeted and targeted datasets. EWAS measures as many chemical entities as possible in samples from different populations without any preconceptions or bias. Simultaneously measuring the totality of chemicals, including those from foods, drugs, pollutants, oxidative stress, metabolites and the activity of the gut microbiota circulating human blood, provides a much larger picture of the chemical environment than measuring one “ome” at a time. In this way, EWAS seeks the non-genetic causes of chronic diseases much in the same way GWAS explores genetic causes. Of course, no one analytical approach can measure everything, but by leveraging multiple techniques such as LC-MS, ICP-MS and GC-MS, one can map large swaths of the exposome space. Herein, EWAS with high resolution, accurate mass in the gas phase yielded many potential chemical entities such as normal metabolites, dietary chemicals and many unidentified chemical features (e.g., an accurate mass, consistent peak shape of several extracted ions at a specific retention time and an abundance > then the defined threshold). POPs in the plasma samples were putatively identified in the GC-QTOF data in two-fold manner: first, post-analysis chromatographic deconvolution extracted chemical features from the datasets. This was followed by statistical analysis and spectral library search of the features using MPP. To validate the EWAS findings, targeted GC-MS/MS for 15 polyaromatic hydrocarbons, 12 dioxin-like polychlorinated biphenyls, 11 polybrominated diphenylethers, 18 organochlorine pesticides, 5 dioxins and 5 furans was performed. POPs concentrations are 1000-fold lower than chemicals derived from food, drugs and metabolites. Most non-targeted analyses fail to detect approximately 70% of these compounds; however, GC-MS/MS technologies are especially well-suited to measure these in biological systems at parts-per-trillion concentrations. Qualitative differences between populations with dissimilar POPs exposure profiles were determined with secondary statistical analysis filtering by abundance / frequency, unpaired t-test for case vs. controls and one-way ANOVA for case/controls vs. other variables. GC-QTOF EWAS followed by targeted GC-MS/MS can identify and validate markers of exposure in human plasma at parts-per-trillion concentrations

POSTER 174

The Application of UHPLC-Orbitrap MS in Trace Level Untargeted Screening of Bisphenol A Analogues and Derivatives in Human Urine

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Bisphenol A (BPA) has been shown to have endocrine activity and may affect the fetus and infant, including possibly causing changes in development and behavior. California enacted legislation banning the use of BPA in baby bottles and sippy cups, which was effective July 1, 2013. Also, as of July 2012, the U.S. Food and Drug Administration permanently ended the use of BPA in these products. Manufacturers have been considering BPA analogs or derivatives (BPAAD) as replacements for BPA in various applications. However, BPA analogs or derivatives may leach into food or be transferred from paper products, leading to human exposure. Untargeted screening in human urine can provide an indication of human exposures to these emerging compounds. In the present study, 20 microliters of a pooled human urine sample were injected into an ACE Excel 2 C18 PFP UHPLC column (150x2.1mm, 2 micron) with a general gradient of 0% to 100% methanol (B) located on a Dionex Ultimate 3000 UHPLC coupled to a Thermo Exactive Plus orbitrap MS ionized in electrospray source and operated in full scan mode, then data were analyzed with an accurate mass filter of less than 5 ppm in Thermo TraceFinder software which was linked to an in-house built library specifically to include all of the currently available BPAAD compounds, and, in addition, more than 600 other high priority persistent organic pollutants (POPs) throughout thorough literature search. Peak shape, signal to noise ratio, accurate mass, isotope pattern were used as criteria for potential feature hits of interest. More than ten hits of interest existed at low levels in the pooled urine sample, most of them were BPAADs, three were phthalates, one was PFA, one was herbicide, and two were musk oil components for cosmetics. Later we

also tested matrix effects of the Exactive Plus MS by injecting pooled urine samples spiked with certain amount of specific BPAADs (BADGEs, BFDGEs, etc) at different levels, our preliminary results suggested that the Exactive Plus MS was not optimal at handling complex matrix. Therefore, we upgraded our instrument to the Quadruple-Exactive Plus with a HCD collision cell. We will continue studying how good Q-Exactive is at reducing the effects of complex matrix. Our research may have a significant impact on future directions of the human biomonitoring program in the State of California and those in the United States. Trace level untargeted screening of high priority environmental contaminants in human biofluids is possible with our current workflow.

POSTER 178

Detection and analysis of phase II synthetic pyrethroid metabolites in the urine of farming communities of Western Australia

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The use of pesticides is a considerable component of the crop management systems employed by most of the farming communities of Western Australia. Synthetic pyrethroids, man-made analogues of naturally occurring pyrethrins, are the most commonly used pesticides within commercial household products and agriculture. Pyrethroids undergo phase I metabolism yielding 5 common metabolites for the 12 most widely used pyrethroids in Australia. Subsequent phase II metabolism predominantly conjugates these metabolites to a glucuronide unit to facilitate excretion in the urine. Arguably, of highest risk of exposure to these pesticides are the farmers administering them and potentially, their families. Methodology was developed to analyse the phase II metabolites using high resolution liquid chromatography-mass spectrometry to monitor pesticide exposure. A quantitative method for the analysis of phase II metabolites in human urine was developed using a Sciex 5600 QToF-MS. Commercially available phase I metabolites, including cis/trans-3-(2,2-dichlorvinyl)-2,2-dimethylcyclopropane carboxylic acid (DCCA), cis-3-(2,2-dibromovinyl)-2,2-dimethylcyclopropane carboxylic acid (DBCA), 3-phenoxybenzoic acid (3-PBA), labelled 3-phenoxybenzoic acid (13C6-3-PBA) and 4-fluoro-3-phenoxybenzoic acid (F-PBA) were purchased and glucuronidated in-house using human liver microsomes. Quantitation of phase II metabolites was possible based upon the observed concentration difference in phase I metabolites after glucuronidation. Pesticide metabolite levels were also normalised to creatinine content. Mass spectral data was collected using electrospray ionisation in negative ion mode for the pyrethroid metabolites and positive mode for creatinine analysis. Additionally, MSMS spectra were obtained for all phase I and phase II precursor masses. All samples underwent solid phase extraction (SPE). Extraction efficiency was tested by spiking urine samples both before and after SPE, and recovery ranged from and 107-122% for phase I metabolites and 90.8-99.7% for the glucuronide products. Conversion of phase I to phase II metabolites using human liver microsomes yielded 89.6% DBCA-Glc, 86.4% DCCA-Glc, 37.8% F-PBA and 36.8% PBA-Glc. Due to the complex nature of urine matrices, both phase I and II metabolites showed significant ion suppression. Phase I metabolites were suppressed in the range of 36.1-54.2% and the glucuronide analogue suffered greater at 41.0-79.4% suppression of signal. Method detection limit for the analysis of phase I metabolites in human urine ranged from 0.2-1.1 ng/mL and 0.6-1.3 ng/mL for the glucuronides. A total of 94 urine samples were collected from 15 farmers, 17 spouses and 15 children both before and two weeks after a pesticide spray event. Incidences of all four phase II metabolites occurred, with F-PBA-Glc being detected in 8.3% of samples, DBCA-Glc in 21.1%, 3-PBA-Glc in 81.7% and DCCA-Glc in 85.3% of all samples. There was no detection of any phase I metabolites. No significant difference was seen in detected levels between the initial sample collection and the subsequent collection, potentially indicative of post spray event sampling occurring too far from pesticide application. An observed difference was seen between the children and parents with the children presenting higher levels of pesticide metabolites within their urine at both time points. LC-QToF-MS analysis of phase II metabolites; pesticide exposure to farming families.

POSTER 179

Dissolved organic matter profiles of a grassland soil surrounding a fall rainfall event

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Dissolved organic matter (DOM) is an important component of the terrestrial ecosystem. As a major source of substrates for soil microbial communities, DOM composition can impact microbial speciation, activity and thus, ecosystem biogeochemical cycles. Despite the significance of soil DOM to terrestrial ecosystems, DOM composition and fluxes are still not well understood in soils. In Northern California, precipitation typically varies by season with relatively wet winters and dry summers which lead to periods of rapid decomposition initiated by fall rainfall events. These discrete rainfall events permit a distinctive opportunity to examine DOM turnover in fine detail. Grassland soils that were collected from the Angelo Coast Range Reserve (Branscomb,CA,USA) on 4 dates from August 20th, 2013 and October 2nd, 2013 in 10 cm increments from 0 to 40 cm. Water soluble compounds were extracted from the soils using cold LC-MS grade H2O, and the extract was then lyophilized and analyzed on an Agilent 6550 iFunnel Q-TOF LC/MS equipped with a zwitterionic ZIC-pHILIC HPLC column (EMD). Relative (putative) metabolite concentrations were determined using Metabolite Atlas. Metabolite Atlas is a pipeline for analyzing and distributing LC-MS/MS metabolomics data that is currently in development by a group led by Benjamin Bowen, NERSC and the Northen Lab at LBNL. As part of an integrated, multi-omics research project, polar- hydrophilic-compounds were extracted and analyzed from the top 40 cm of grassland soil before and after the first two fall rainfall events at Angelo Reserve in September 2013. Over 140 compounds including a number of verified and putative metabolites where detected and tracked at 4 soil depths over. Most of the compounds detected in our analyses were simple sugars (1 to 6 sugar residues) and sugar alcohols. Additional compounds detected include: sugar-derivatives, nucleotides/nucleosides, sterols, quaternary ammonium compounds and amino acids. Prior to rainfall, the relative concentrations of DOM species decreased rapidly with depth. Following the first rainfall, the majority of compounds analyzed decreased in relative abundance in the top 10 cm. A subset of sugars and other compounds temporarily increased in relative abundance in the lower levels, particularly from 10 – 20 cm, before being depleted. Following the second rainfall, several of the detected compounds increased in relative abundance at all measured depths resulting in increased amounts of several amino acids, nucleotides/nucleosides and alterations in sugar species. This research uses LC-MS/MS analyses of polar soil metabolites to track metabolites through the upper soil column over time.

POSTER 180

DIFFERENTIAL METABOLOMIC PROFILING OF MUSSELS AND PASSIVE SAMPLERS FOR UNTARGETED SCREENING OF ALGAL TOXINS IN SEAFOOD AND COASTAL MARINE ENVIRONMENTS

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Microalgae known to produce biotoxins may accumulate in fish and shellfish, and render these products harmful to seafood consumers. Globally, monitoring programs rely on phytoplankton and shellfish surveillance for consumer protection. Passive samplers have shown their ability to accumulate biotoxins produced by toxic microalgae directly from seawater. Additionally, extracts of passive samplers are assumed to be less complex matrices than shellfish, which may rapidly metabolize algal toxins and also

contain endogenous compounds. Matrix effects were evaluated for mussel and passive sampler extracts and compared using low (LRMS) and high resolution (HRMS) mass spectrometers. In addition, a differential metabolomic approach was used to investigate and compare the chemical diversity of passive samplers with those of mussels from different French coastal areas. Passive samplers were deployed for 1-week periods at Ingril lagoon, Concarneau and Villefranche-sur-mer. For comparison of toxin profiles, mussels were also deployed at Villefranche-sur-mer, and native mussels were used for Ingril lagoon. All samples were extracted with methanol. Blank extracts of mussels and passive samplers, as well as methanol, were spiked with lipophilic phycotoxins to generate matrix-matched and methanol calibration solutions. Those solutions were further analyzed using HPLC/UHPLC systems connected to either an API4000TM (AB Sciex), an ExactiveTM (ThermoFisher Scientific) or a Quadrupole Time-of-Flight (QTof Agilent) mass spectrometer. This latter instrument was also used to evaluate metabolite profiles of field samples. Differential profiling was carried out using Agilent MassProfiler Professional™ and MassHunter Qualitative B.06™ software packages. Full-scan spectra from mussel extracts showed more co-eluting compounds at toxin retention times than equivalent spectra from passive sampler extracts. However, these co-eluting compounds were of significantly different masses than the toxins of interest, hence they did not result in mass interference in high resolution spectra. Therefore, matrix effects should be related to ionization phenomena in the source rather than at detector level. Additionally, comparison of methanol and matrix-matched calibration curves acquired using either LRMS or HRMS showed that passive samplers quantitatively generated less matrix effects than mussel samples. This observation was true for all toxins spiked and for HRMS it was independent of the mass spectrometric detector (Q-ToF or orbitrap). Hence, both qualitative and quantitative observations pointed clearly to matrix effects being mainly related to ionization phenomena in the source. The comparison between mussels and passive samplers from the same geographical region using principal component analysis (PCA) clearly showed different compounds in these two matrices. Passive samplers deployed in different field locations were also easily distinguished by PCA. Similarly, passive samplers deployed at different dates (early, medium and late deployment stage) at the same location were also differentiated by PCA, reflecting seasonal variations in the phytoplankton community. Identification of discriminating compounds is ongoing using marine natural products and MetLin™ databases as well as an in-house developed database for algal and cyanobacterial toxins. The reduced matrix effects for passive sampler extracts facilitated statistical analysis allowing sufficient simplification of metabolomic data in order to interpret temporal and spatial differences of toxin profiles. These techniques also contribute to a clearer picture of emerging organisms and toxins in coastal waters. This study used a metabolomic approach for spatial and temporal differentiation of marine environmental toxin profiles using passive samplers.

POSTER 181

Applying comparative metabolite profiling to understand complex cellular mechanisms in the coral-dinoflagellate symbiosis

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Metabolic exchange is central to the ecological success and stability of the coral-dinoflagellate symbiosis and may also play a role in host-symbiont specificity. However little is currently known about this complex nutritional relationship, including how different algal symbionts affect the metabolite pools of the same cnidarian host, particularly during novel associations. Comparative metabolite profiling of has the potential to greatly enhance our knowledge of symbiosis-dependent metabolism and photosynthate translocation from symbiont to host. It is of paramount importance to develop a protocol to separate the endosymbiont from host tissues that adheres to the time and practical constraints inherent of metabolomic sample preparation whilst providing reliable and reproducible information. We have developed and applied a protocol to the anemone host (Aiptasia pulchella) in symbiosis with their homologous (natural occurring) and heterologous (novel) algal symbiont types (Symbiodinium spp.). These represent a diverse physiological range (e.g. thermal tolerance) in the hope of revealing the nutritional potential of the

complex coral-dinoflagellate symbiosis and its chances of survival in a changing climate. Metabolites were extracted from both host and symbiont fractions before separation into hydrophilic and lipophilic fractions. The metabolites were chemically derivatized and analyzed using Gas Chromatography separation linked to Mass Spectrometry (GC-MS). Qualitative and semi-quantitative multi-component analyses were used to describe metabolic patterns for metabolically- or analytically-related compound; in this case photosynthetic products translocated from the dinoflagellate symbionts to the host. Our method is currently being used to generated a comprehensive profile of hydrophilic and lipophilic compounds to provide an insight into the nutritional status of the host cnidarian, and hence the potential for survival and proliferation when in symbiosis with different symbiont types. GC-MS analysis is currently underway, and preliminary data shows a variation in the diversity and abundances of fatty acids in heterologous associations in comparison to their homologous counterparts. Relative abundance of important structural fatty acids such as 22:6(n-3) also appears to differ between the symbiont types. Once analysis is complete, we hope to identify the role of metabolism in host-symbiont specificity, highlighting potential compounds that influence the formation of novel pairings and the ability for corals to adapt to a changing climate. Our method was applied to the anemone A. pulchella, which is recognized as a model system in cnidarian-dinoflagellate symbiosis research, but will further be addressed in coral systems. Optimised sample preparation for metabolite analysis of a symbiotic system; applied metabolite profiling to help understand complex endosymbiotic interactions

POSTER 182

Deep Metabolome Annotation of the ecotoxicological and NIH model organism, Daphnia magna (the water flea).

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Technological, methodological and computational innovations have, in recent years, led to a marked expansion of the metabolomics platform and it's possible areas of application. Inefficiencies of metabolite annotation and identification, nevertheless, continue to hinder derivation of meaningful biological inferences from many metabolomics datasets. Multiple-stage mass spectrometry (MSn) offers a route by which to address this critical challenge, both through improved empirical formula assignment and through aiding in structural elucidation efforts; however, few groups currently exploit it's full potential. Here we describe an enhanced workflow, coupling extensive physico-chemical separation, metabolome fractionation and optimised MSn acquisitions, for the Deep Metabolome Annotation of the ecological, ecotoxicological and NIH model organism, Daphnia magna (the water flea). Ten strains of Daphnia magna were maintained under defined and multiple culturing conditions prior to metabolic quenching. A single pooled homogenate was generated and independent polar and apolar metabolite extractions performed. Using complementary solid-phase extraction (SPE) sorbents, polar and apolar metabolome components were fractionated via coarse physico-chemical interactions. Resulting SPE fractions were further separated via a minimum of three complementary liquid chromatography-mass spectrometry methodologies, each in both positive and negative ionisation modes, with concurrent collection of mass spectral data and time-based chromatographic fractions. After computational filtering, LC fractions were submitted for multiple-stage mass spectral analyses (MSn) on hybrid Orbitrap platforms, with dynamic spectral acquisition at maximum resolution, under multiple collision modes and energies, for all features in a given fraction. In collaboration with scientists from Thermo Scientific (as part of our Technology Alliance Partnership), a workflow has been developed to facilitate the extensive fractionation of the Daphnia magna metabolome. This workflow applies SPE and LC-MS approaches, prior to MSn analysis of multiple metabolites present in each LC-MS fraction. We have applied this procedure to significantly advance the current status of Daphnia magna metabolome annotation that, at the start of 2015, totalled fewer than 130 reported metabolites of varying annotation quality. As a first step in workflow development, relevant physico-chemical interactions, literature and experimental outcomes were considered in defining four complementary SPE protocols for the coarse fractionation of metabolome components; two each for polar and apolar metabolome extracts, each yielding a minimum of three

distinct fractions. A total of three complementary LC columns were applied to the separation and fractionation of both polar and apolar SPE fractions; each LC method was optimised through changes in, for example, solvent eluotropic strength, pH, buffer type and concentration. In our results to date, we have demonstrated the efficiency of metabolome detection applying two different methods: (1) for the apolar arm, we have demonstrated the detection of >1000 unique lipid species, in each of three C18 SPE fractions, during reversed-phase LC-MS/MS analyses in the positive ionisation mode; (2) for the polar arm, we have demonstrated the outcomes from optimisation of our phenyl-LC-MS methodology, as implemented for polar metabolome separations, in which at least an 8.2% increase in reproducible feature detection was achieved (13050 vs. the non-optimised method with 12052 features); an exciting output from a workflow including five complementary LC column chemistries. We will present the Deep Metabolome Annotation workflow, descriptions of the optimisations of the LC-MS methods including fractionation, and proof-of-principle data from MSn acquisitions of the fractionated metabolome components. An unparalleled workflow combining extensive physico-chemical fractionation with multiple-stage mass spectral analysis, for deep metabolome annotation.

POSTER 183

Metabolomics approaches for biomarker discovery for toxicant exposure of di-isononyl phthalates (DINPs) using liquid chromatography-high resolution mass spectrometry (LC-HRMS)

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Di-isononyl phthalate (DINPs) is a kind of phthalates which are widely used in plastics, building materials, toys, and personal care products. Exposure assessment of DINPs in human is of interest because of the potential adverse health effects of DINP. However, our knowledge on DINP metabolism remains limited. The development of high-resolution mass spectrometry (HRMS) instrumentation such as FT ion cyclotron resonance and Orbitrap, along with new data processing techniques, has improved the quality and productivity of metabolite identification process. In the present study, we explored three metabolomics approaches for DINPs exposure marker discovery using the LTQ/Orbitrap HRMS and multiple post-acquisition data processing techniques, including signal mining algorithm with isotope tracing (SMAIT), mass defect filtering (MDF), and on-line web-based software XCMS. The MMOP standard was incubated with liver enzymes to generate DINP metabolites and LTQ/Orbitrap HRMS coupled with three data processing methods (SMAIT, MDF, and XCMS) to perform mass spectral signal filtering in a complex LC-HRMS data obtained from DINP metabolites. For SMAIT strategy, D0-MMOP and D4-MMOP mixtures with varying isotope concentration ratios were used; for MDF and XCMS strategies, one concentration of D0-MMOP standard was used. The probable metabolite signals filtered from these three strategies were further evaluated as DINP exposure markers by a rat model. The rat urine samples were collected from the rats administered with several doses of commercial DINP or corn oil (carrier). These probable metabolite signals were detected in rat urine samples using LTQ/Orbitrap. There were 16 probable DINP metabolite signals filtered by SMAIT, 84 by MDF and 139 by XCMS. Total of 189 probable DINP metabolite signals were filtered by the three strategies. Due to the reasonable number of signals filtered by SMAIT, 16 probable metabolite signals were validated as DINP exposure markers using a rat model. Out of the 16 probable metabolite signals, 14 signals were validated as effective exposure markers because of the established dose-response relationship. Among the 14 DINP exposure-related signals, 8 have not been reported in the prior literature. The chemical structure of the probable metabolite can be elucidated by MS/MS product ion profile and accurate mass measurement. In a previous report, the DINP metabolite signal m/z 293.1 was identified as mono(carbonyl isohexyl) phthalate (m/z 293.103). However, we hypothesized that it could be mono(hydroxyl isooctyl) phthalate (m/z 293.139) as suggested by accurate mass measurement, relative isotopic abundance, and data derived from a synthesized standard. This DINP metabolism data can provide valuable information for further study on DINP toxicity, exposure assessment, kinetics, and human health effect. Propose a metabolomics platform can efficiently filter probable metabolite signals from a complex LC-HRMS dataset for toxic exposure marker discovery.

POSTER 184

A nanoLC-HRMS method for untargeted analysis of small-molecule exposomes in archived dried blood spots

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The analysis of dried blood spots (DBS) offers an important tool for biomarker discovery in epidemiological studies. Since neonatal DBS have been archived in California and some other states, they represent an important resource for characterizing exposures at birth that may be determinants of diseases occurring in childhood and later life. DBS are also simpler to obtain and archive from adolescent and adult populations than venous blood. Thus, untargeted methods for measuring small molecules in DBS would be very useful for etiologic studies of human diseases. Here we compare metabolomes derived from 4-mm DBS punches with various organic extraction and precipitation systems and analyzed with nano-liquid chromatography (LC) – high resolution mass spectrometry (HRMS). Whole EDTA blood was spotted on Whatman 903 filter paper, dried under vacuum, then stored at -20°C. Four-millimeter punches from the DBS and adjacent filter paper were extracted and precipitated with 7 different combinations of ethanol, methanol, and acetonitrile in water and stored at -80°C prior to analysis with nanoLC-HRMS. Samples were injected into a nanoflow microfluidic chip (Chip Cube Ò, Agilent Technologies), which integrates a trapping and analytical C18 column, coupled with a QToF HRMS (Model 6550, Agilent Technologies). Mass spectra were acquired between 50-800 m/z in positive-ion mode. Peak picking, retention-time alignment, preprocessing, outlier removal, and annotation were conducted using software coded in R More than 20,000 features were detected in the untargeted analysis of DBS extracts. After filtering for features with coefficients of variation (CVs) less than 30%, fold-change analysis and t-tests detected between 1000 and 4000 features that were blood specific, i. e. present at significantly higher concentrations than observed in extracts of blank-filter punches (family-wise P-value of 0.05 after correction for false discovery rate). Extraction with acetonitrile yielded the highest number of overall features and the highest number of unique features compared to the other extraction systems. A recently-developed bioinformatics algorithm (CompositeMS2Explorer) was used to match the MS feature table to the precursor ions of data-dependent MS/MS fragmentation based upon agreement of retention times and accurate masses. Untargeted MS/MS fragmentation data within the run produced >1200 MS feature matches to composite spectra. Over 150 metabolites representing biologically relevant entries were annotated with HMDB with composite MS2 spectra matched to HMDB or Metlin. Annotated features ranged from polar to non-polar with estimated log D values from -0.5 to 12 at pH 2. Not surprisingly many annotations represented lipid classes including phosphocholines, lysophosphatidylcholines, sphyingolmyelins, and acylcarnitines. Examples of annotations include caffeine, glycine conjugates of linolenic acid, and PE(P-16:0e/0:0). The dynamic range of the method was investigated by quantifying selected features extracted from different amounts of DBS material ranging from ¼ of a 4 mm punch to a whole 4 mm punch. Results indicate that untargeted small-molecule exposomes analysis can be performed with a 4-mm DBS punch, representing ~5 mL whole blood.

POSTER 185

METABOLIC PROFILING OF ZEBRAFISH EMBRYOS EXPOSED TO BISPHENOL-A

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Zebrafish (Danio rerio) is a model organism for environmental risk assessment of chemicals due to their rapid development into larvae (within 48 hours) and to their high sensitivity to chemical treatments. In addition, its small size and easy growth conditions offer the possibility to perform small-scale analyses for -omics studies, including metabolic profiling. Bisphenol A (BPA) is a chemical produced at large-scale for

use in the production of plastics and epoxy resins. Furthermore, BPA is an endocrine disruptor that interferes in the endocrine system of aquatic biota at concentrations often found in certain ecosystems. In this work, the effects of bisphenol A on metabolic profiles of five-day-old zebrafish embryos is evaluated. Zebrafish embryos were exposed to different BPA concentration levels of BPA (1, 2 and 4 µg/mL which is the LOEC, Lowest observed effect concentration). Then, an untargeted metabolomic approach coupling chromatographic separation by hydrophilic interaction liquid chromatography (HILIC) and mass spectrometric detection was used. Advanced chemometric tools were used to evaluate the changes in the metabolome between zebrafish embryos maintained at standard conditions and embryos under different BPA concentrations. A combination of principal component analysis (PCA), partial least squares discriminant analysis (PLS-DA) and multivariate curve resolution alternating least squares (MCR-ALS) was used to detect the metabolites related to the exposure induced changes. Initial exploration of Total Ion Current (TIC) chromatograms by PCA allowed the classification of the samples according to the BPA exposure, especially in the case of considering negative ionization MS mode. However, relevant information could be missed by just studying TIC chromatograms as significant minor contributions may not be considered. For this reason, an analysis workflow based on the MCR-ALS method was used in order to retrieve this information, resolve overlapped electrophoretic peaks and provide the elution and mass spectra profiles of the detected metabolites. Only MCR-ALS resolved elution profiles that showed a significant difference between the groups of samples were then selected for further analysis. This strategy allowed reducing the number of potential metabolites to approximately one hundred. After that, the accurate mass of these candidate metabolites and their fold-change according to BPA exposure was determined. Statistical significance of the obtained metabolites was assessed by a post hoc corrected ANOVA model. Finally, identification of the metabolites was attempted by searching in on-line resources. Information regarding the metabolic pathways and mechanisms affected by the BPA exposure was also recovered. Preliminary identification of the metabolites and of the metabolic pathways involved in the BPA exposure seemed to influence the metabolism of amino acids and organic acids of zebrafish embryos. This work was supported by the European Research Council under the European Union's Seventh Framework Programme (FP/2007-2013)/ERC Grant Agreement n. 32073. Combination of HILIC-HPLC-MS and multivariate data analysis tools for zebrafish embryos metabolic profiling.

POSTER 186

Metabolic profiling of daphnia magna from gas chromatography-mass spectrometry multivariate curve resolution data analysis

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Daphnia magna is a model organism used for environmental toxicological assessment in aquatic media. Metabolic profiling is required for achieving the most extensive possible range of compounds and of their concentration changes in stressing conditions. However, data processing is a challenging step and mathematical tools are needed to allow extracting the required information on metabolic changes. Multivariate curve resolution-alternating least squares (MCR-ALS) is proposed for the resolution of complex chemical mixtures, and estimate their pure constituent response profiles. MCR-ALS is a powerful data analysis tool that solves the co-elution problem of complex natural samples as well as problems derived from gas chromatography-mass spectrometry (GC-MS) systems, such as baseline drift, spectral background, noise contributions or low S/N ratio values. D. Magna was exposed to an increase of salinity of their normal conditions. Polar metabolites of the whole organisms were extracted, derivatized twice and analysed by GC-MS. Data were recorded in full scan mode to evaluate the effect of salinity on the Daphnia metabolome. Original GC-MS full scan data of control and exposed samples were transferred into a Matlab environment. Every chromatographic run of every sample were arranged in a single individual data matrix and they were reorganized together in a column-wise augmented data matrix (samples x elution times, m/z values). This augmented data matrix was subdivided into 11 distinct chromatographic regions along the elution time, giving 11 new augmented data matrices which were then resolved by MCR-ALS. Due to the high fragmentation of the molecules observed in GC-MS, to the high

number of overlapped (co-eluted) peaks and to the presence of a large number of derivatizing subproducts, the analysis by MCR-ALS results to be extremely helpful because it permits the discrimination among elution profiles of Daphnia relevant metabolites and the those from the large number of interferents (the large number of undesired derivatized compounds) and to identify (via their MS spectra) the more relevant metabolites. A total of 81 MCR-ALS components were resolved and a statistical analysis was applied to identificate possible metabolic biomarkers among them. Results from a discriminant partial least squares (PLS-DA) analysis permitted to identify a final number of 66 metabolites, which could be proposed as biomarkers. The tentative metabolite identification of these 66 metabolites was carried out through the NIST 2014 mass spectral reference library and the utilization of Golm metabolome database, from which the most important altered metabolite pools caused by the studied pressure in Daphnia metabolome is proposed. Most of the components were identified with the high Relative Matching Factors (RMF) values and to increase the reliability of the identification, the retention index (RI) in the evaluation of the library hits was also used. For each mass spectrum, 100 hits were retrieved. As a preliminary conclusion, the increase of salinity is shown to affect significantly carbohydrate and amino acid levels. MCR-ALS is confirmed to be an insightful tool for metabolic profiling of intrinsically complex GC-MS datasets.

POSTER 187

Bioactive compounds from Bees: Identification and Quantitation of Metabolites in Propolis extracts from different sites in Germany

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Propolis is a natural resin collected by bees from the buds of selected plants. Its ingredients show a large variety of remarkable biological activities, for instance against microorganisms. Bees use these activities for a protection of their hives. In the recent 20 years, the interest in this natural product has grown immensely, as propolis is used as a natural medicine or as a food supplement. More than 200 scientific papers published per year in 2013 and 2014 (PubMed) clearly illustrate this increased interest. In this study, samples from seven different bee yards were collected, analysed via LC-MS and investigated in a statistical profiling experiment. The main compounds of interest were identified and quantified. Propolis samples were extracted with 70% EtOH using an ultrasonic bath. The extracts were centrifuged and subsequently analyzed by hr LC-MS (Bruker Impact II QTOF-MS and Thermo Scientific Dionex RSLC). Each sample was run in triple injections. The molecular features were extracted automatically, filtered and binned to create a bucket table. Based on this input, a principal component analysis was calculated to identify differences in the composition of samples (ProfileAnalysis, Bruker Daltonics). Identification of metabolites was done using the exact masses and isotopic pattern information of MS and MSMS data. Database queries were done using the CompoundCrawler, followed by evaluation against in-silico generated fragments. (SmartFormula3D and MetFrag [1]). Quantitation of selected compounds was done using the TASQ software (Bruker Daltonics). All propolis samples showed a general chemical similarity (European poplar type of propolis). But nevertheless, the PCA analysis revealed several compounds which exhibited significant differences in the concentration levels. Exemplary, four of these as well as six of the main compounds were further investigated. The respective elemental compositions were calculated using the measured exact masses and isotopic patterns. The formulae were then refined using the MSMS fragment information. An alignment with “in-silico generated fragments” confirmed in most cases one likely chemical structure. The final verification was done by a comparison with the retention times, LC-MS and –MSMS data of the pure reference standards. The investigated compounds were cinnamic acid, Poster coumaric acid, caffeic acid, ferulic acid, chrysin, galangin, caffeic acid phenethyl ester (CAPE), quercetin, naringenin and apigenein. All of them are known to be ingredients in poplar type propolis. The quantitation of the ten metabolites was done using the peak areas of the hrEIC traces ([M+H]+ adducts). Identification and Quantitation of propolis components using LC-MS and –MSMS data.

POSTER 188

KNApSAcK Metabolite Ecology Database for Investigating the Relationships Between VOCs and Biological Activities

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Volatile organic compounds (VOCs) are small molecules with low molecular weight and have low boiling points. Although VOCs comprise only a small proportion of the total number of metabolites produced by living organisms, however, because of their important roles in chemical ecology, specifically in the biological interactions between organisms and ecosystems, revealing the roles of these VOCs is essential for understanding the interdependence of organisms. Information on volatile emission from living organisms is scattered in the literature, but there is no database that accumulated information on species-species interaction and biological activities of VOCs until now. To attain this purpose, we have developed a KNApSAcK Metabolite Ecology Database, which contains the information of VOCs and their biological activities. The data were accumulated from an extensive literature search available on PubMed and Google Scholar. The information on VOCs, emitting species, target species and their biological activities were extracted and deposited into a KNApSAcK Metabolite Ecology Database. In order to investigate the relationships between VOCs and biological activities, we determined the Tanimoto similarity measure between two chemical compounds using ChemMine package in R. Then, heat map clustering was performed for classifying the VOCs based on chemical structure similarity. We also determined the Poster values of the clusters based on hypergeometric distribution to relate a structure group to a biological activity if and only if the structure group is overrepresented by VOCs with that biological activity. At present, we have accumulated 1088 VOCs emitted by 517 microorganisms species and 341 VOCs emitted by other biological species including plants, animals and human. These VOCs data have been deposited into a KNApSAcK Metabolite Ecology Database, which allows users to search information on VOCs using the KNApSAcK compound ID and metabolite name. This KNApSAcK Metabolite Ecology database is also linked to the KNApSAcK Core and KNApSAcK Metabolite Activity Database to provide further information on the volatile metabolites and their biological activities. The VOC database can be accessed online at http://kanaya.naist.jp/MetaboliteEcology/top.jsp. The biological activities of VOCs are divided into two types: (i) chemical ecology related activities, in which most VOCs involved in the interaction between species for survival of organisms such as defense and antimicrobial, (ii) human health care related activities, in which many VOCs are widely used as disease biomarker and odor. From our accumulated data, 57.3% of the activities belong to chemical ecology such as antifungal, antimicrobial, attractant, defense, enhance plant growth, inhibit root growth and repellent activities and 42.7% are human health related activities such as disease biomarker, odor, anti-cholinesterase and antioxidant. From the heatmap clustering result, we tentatively classified 11 clusters of VOCs with various chemical groups and biological activities. All VOCs belong to Cluster 1 and Cluster 2 are terpenoids chemical groups, in which the main activities are antimicrobial and defense activities. VOCs classified into Cluster 3 and Cluster 4 are alkanes and alkenes respectively with disease biomarker as their main biological activity. The heatmap clustering result shows that there are strong links between chemical structure of VOCs and their biological activities. Comparative activity relationships between chemical ecology and human healthcare activity will lead to systematization of metabolomics combined with human and ecological metabolic pathways. KNApSAcK Metabolite Ecology database is the first database, containing the information on species-species interaction based on VOCs and biological activities.

POSTER 189

Environmental Metabolomics and Ecotoxicity Modeling using Earthworms

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Advancing the understanding and monitoring of environmental health is dependent on the advancement of analytical technologies and ecotoxicity evaluation. Current limits of detection strain true characterization of chemicals in the environment, some of which may hold significant toxicological implications at their low, unquantifiable levels. Thus, focusing efforts on alternative ways to identify contaminants or ecotoxicity is necessary, such as with metabolomics. Understanding how organisms respond to toxicants and the toxicometabolome is key in order to begin using metabolomics as a biomonitoring tool to assess environmental health. Here, earthworms (Eisenia fetida) are used as an ecotoxicity model to evaluate the metabolomic impacts of toxicants, including dose-response and bioaccumulation, and the potential of metabolomics as an analytical tool to evaluate environmental

health. This work anticipates to develop an ex situ soil microcosm to mimic chemical transport and evaluate exposure. Proton and 2-D nuclear magnetic resonance is used in tandem with mass spectrometry to characterize the metabolomic impact of contaminants on earthworms, in addition to analyte concentration within tissues and environmental. Preliminary results have demonstrated NMR as a useful tool to fingerprint the metabolome of earthworms and their coelomic fluid – a biofluid useful for complimentary metabolomic assessment. Current research is moving towards developing methods for metabolomic characterization of earthworms and coelomic fluid using mass spectrometry. This research aims to model metabolomics as an ecotoxicity assessment tool, by evaluating how environmental relevant doses effect metabolomic response.

POSTER 190

Personalized and Baseline Environmental Health of Human Managed Bottlenose Dolphin through Metabolic Profiling

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The National Institute of Standard and Technology (NIST) collaborates with Dolphin Quest (Hawaii) to assess environmental and anthropogenic stressors that may impact the long-term health of bottlenose dolphins. Bottlenose dolphins are considered a good sentinel species for coastal ecosystem health and human exposures to contaminants in seafood due to some of their life history traits, such as being relatively long-lived mammals and the frequency at which they produce a single offspring at a time, and they share some of the same coastal resources as humans. Captive dolphins research is valuable to address health concerns that effect animals in captivity and also extremely important to aid in answering questions about wild dolphins that a captive controlled setting can provide for research. Metabolomic measurements were made on 133 filtered sera samples from 15 dolphins using Nuclear Magnetic Resonance (NMR). Analyses were made on quarterly blood samples from dolphins of various states including healthy, ill, pregnant and newborn using multivariate and univariate statistical methodologies. Initial analyses are being conducted to assess the quality of the data, the reproducibility of the sera processing technique, and the composition of the field blanks. A preliminary profiling of the dolphin sera has resulted in annotation of approximately 60 known metabolites. Two dimensional NMR experiments have been run to aid in confirmation of these annotations, as well as, to provide additional data to assist in the identification of unidentified metabolites. Establishing metabolic profiles for bottlenose dolphin in various health state and also establishing baseline health profiles for wild caught dolphin.

POSTER 191

1H NMR-based Metabolomics to Study Effects of Solvent Exposure on Workers from Synthetic Leather Factories

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In the manufactoring processes of synthetic leather, the workers might expose to variety of solvents, such as dimethylformamide, toluene, methyl ethyl ketone which are all confirmed to have adverse health effects toward human by previous studies. This reasearch is to study metabolic effects of numerous co-exposure solvents on the workers of synthetic leather factories by using 1H nuclear magnetic resonance (NMR)-based metabolomic approach. Biological samples, urine, were collected from workers (n=39) of a synthetic leather factory in one day at 2 time points, pre-shift and post-shift. Metabolic profile of each sample was analyzed by 500 MHz NMR. Environmental samples were also collected using personal air-sampling to evaluate ambient concentration of dimethylformamide, toluene and methyl ethyl ketone. Grouping criteria were based on the solvent concentration and classified into high (2nd tertile plus 3rd tertile) and low (1st tertile) exposure group. Principal components analysis (PCA), partial least-squares discriminant analysis (PLS-DA), and logistic regression analysis were used to assess the association

between metabolome and solvent exposure. Currently, we focused on the effects of different ambient solvent concentration on urinary metabolome. The PLS-DA model showed obvious metabolome seperation between pre-shift and post-shift when workers exposed to higher concenration of solvents. Considering the co-exposure effects, PLS-DA score plot showed clear metabolome seperartion between high and low concentration of toluene exposure group when workers exposed to higher concentration of DMF simultaniously. In addition, significant variation on metabolome pattern were also related to higher concentration of DMF and toluene exposure. In this study, we can discover biomarkers and identify potential adverse health effects of solvent exposure.

POSTER 192

The Firefighter Exposome: Comprehensive Urine Metabolite Profiling for Assessing the Impacts of Wood Smoke Exposures

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Among first responders, firefighters suffer a higher incidence of work-related diseases with reduced average life span. Chronic physical stress cannot solely account for the frequency of chronic diseases such as coronary artery disease, leukemia, and cancers of the lungs, brain, and lymphatic system. Recently, localized airborne combustion by-products have become primary suspects in the etiology of occupational illness in firefighters. Indeed, polyaromatic hydrocarbons and methoxyphenols are two abundant components of volatile wood smoke by-product that may exhibit significant toxicity. In this work, dynamic changes in the urine metabolome of firefighters were characterized by capillary electrophoresis-mass spectrometry (CE-MS) and gas chromatography-mass spectrometry (GC-MS) to elucidate the impacts of heat stress and wood smoke exposure during training drills in burn houses. 15 adult male firefighters from 3 municipalities provided 24-hour pre-exposure urine samples and personal questionnaires detailing work history and activities of daily living. For the study duration, participants were strongly encouraged to abstain from the consumption of barbecued or burnt foods, smoking, and major lifestyle changes. Each group was exposed to controlled burns of wood-based materials for approximately 30 minutes in an occupational training structure resembling a residential home while wearing full regulatory equipment. 24-hour post-challenge urine was obtained across three time intervals: 0 – 6 hours, 6 – 12 hours, and 12 – 24 hours. Samples were kept cool in provided ice-packed personal coolers. Once aliquoted, samples were maintained at -80°C before analysis by CE-MS and GC-MS. Despite wearing bunker gear and self-contained breathing apparatus, a significant increase in post-exposure urinary excretion of guaiacols, syringols, resin acids, and polyaromatic hydrocarbons was determined by targeted GC-MS analysis. Also, untargeted characterization of the urine metabolome was performed by CE-MS in order to identify dynamic changes after smoke exposure among over two hundred polar/ionic metabolites. Due to the experimental design, multilevel paired and time-dependent statistical data analysis methods with and without creatinine normalization were compared due to the heterogeneity in apparent exposures, metabolism or excretion rates among firefighters in different study sites. It is hypothesized that some of this variation may relate to an individual's specific duties during firefighting and/or possible anomalies in the use or fit of standard equipment. A large number of unknown metabolites were detected in exposed firefighters – de novo structural elucidation was performed by MS/MS in conjugation with predictive electrophoretic modeling. A number of these currently unidentified compounds may show promise as future confirmatory markers of acute wood smoke exposure. Many compounds demonstrated markedly higher abundance in the initial 0 – 6 hour collection period, prior to re-establishing baseline levels after clearance. Endogenous urinary metabolites significantly affected by the challenge provide unique insights into the physiological ramifications of firefighting, including exposure to chemical hazards, cardiovascular exertion, and thermal stress. Correlations to known metabolic processes indicate specific biochemical perturbations that may contribute to a higher chronic disease risk among firefighters. Necessary mitigation steps and possible health and safety implications with respect to an individual and associated regulatory bodies will also be discussed. First application of

comprehensive metabolomics to firefighter exposure studies to identify novel markers of wood smoke exposure in urine.

POSTER 193

Uptake and toxicological effects of metal nanoparticles from motor vehicles on human lung-derived cell lines

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Motor vehicles can produce and emit metal nanoparticles (NPs) via the exhaust and through wear of metallic components. This has raised concerns about the potential impact of vehicle-related NPOSTER induced effects on human health via inhalation; e.g. ceria NPs are increasingly being used to increase fuel efficiency in internal combustion engines, potentially entering the environment via the exhaust. Currently, there is insufficient information available to allow a thorough risk assessment of these vehicle-derived ambient NPs. Omics approaches enable an unbiased assessment of the molecular responses of cells to NPs, which ultimately could help to identify any mechanisms of toxicity and improve risk assessment. Here we investigated the uptake and toxicity of ceria and iron oxide NPs in lung-derived cells (A549, BEAS-2B). Following environmental characterisation, we studied the effects of synthetic ceria (CeO_2 NPs, with an average size of ca. 8 nm) and three types of commercially available iron oxide (alpha-Fe_2O_3, gamma-Fe_2O_3 and Fe_3O_4, from Promethean Particles Ltd). Eelectron microscopy) was employed to assess uptake of ceria and iron oxide NPs into A549 and BEAS-2B cells. Additionally, SYTOX Green Nucleic Acid Stain, Cell Counting Kit-8 (CCK-8) and lactate dehydrogenase (LDH) assays were used to measure any cytotoxicity. Direct infusion mass spectrometry-based untargeted metabolomics and Agilent array-based transcriptomics (single channel microarrays, human 8 x 60K arrays) were performed to investigate the molecular mode of action. Ceria (CeO_2) and iron oxide (α-Fe_2O_3, γ-Fe_2O_3 and Fe_3O_4) NPs were detected by atmospheric monitoring in roadside sites in London and Newcastle, UK, using a micro-orifice uniform deposit impactor instrument. Their detection emphasises the need to investigate the potential for toxicological effects. Ceria: electron microscopy demonstrated the presence of intracellular ceria NPs within membrane bound endosomes of lung-derived A549 cells. Protein corona formation around ceria was found to promote cellular internalisation via the clathrin-mediated endocytosis pathway. In serum containing media (SCM), cell division and viability were not perturbed following incubation with ceria NPs. Nanoceria had no detectable significant effects on cellular metabolism when in the presence of serum, even at the relatively high concentration of 500 µg/ml. However, in the absence of serum, when no protein corona formed around the NPs, plasma membrane disruption (measured by SYTOX Green Nucleic Acid Stain assay) and significant perturbation of cellular metabolism was observed via direct infusion Fourier transform ion cyclotron resonance mass spectrometry. Iron oxide: α-Fe_2O_3 and γ-Fe_2O_3 NPs in SCM were found to internalise using clathrin-mediated endocytosis and caveolae-mediated endocytosis, whereas the Fe_3O_4 NPs tended to enter cells by inducing plasma membrane disruption. In SCM, the mixture of the three iron oxide NPs (to mimic an environmental exposure) and in particular Fe_3O_4 were found to be toxic to lung-derived cells at a relatively low concentration (ca. 1 µg/ml) whereas α-Fe_2O_3 and γ-Fe_2O_3 alone did not induce toxicity at environmentally relevant concentrations. A half maximal effective concentration (EC50) value of ca. 1 µg/ml was calculated from the concentration-response curves of the CCK-8 and LDH assays for the Fe_3O_4 NP and for the mixture. Using these concentration-response curves and the derived EC50 values, three concentrations (0.001 µg/ml,0.01 µg/ml and 0.1 µg/ml) were selected for metabolomics and transcriptomics studies on BEAS-2B and are currently on-going. Pioneering study of environmentally-realistic concentrations of vehicle-derived metal nanoparticles on lung cell lines measured using a metabolomics approach.

POSTER 194

A non-targeted metabolomics approach to investigate amphibian host responses to chytridiomycosis

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Chytridiomycosis is a globally devastating emerging disease of amphibians that has caused the decline or extinction of roughly 200 species of frogs worldwide. While much research has been done on the disease and the fungal pathogen Batrachochytrium dendrobatidis (Bd), the host tissue metabolic response to disease remains poorly understood. We used a gas chromatography/mass spectrometry approach for non-targeted examination of metabolic perturbations to investigate physiological changes associated with time since exposure, and population variation in response to chytridiomycosis. We sampled both skin (site of infection) and liver tissues of 61 alpine tree frogs (Litoria verreauxii alpina) from three populations (two long-exposed and one naïve to the fungus) at various time points (4, 8, 14 days post exposure and moribund) during an exposure experiment involving both exposed (infected) and negative control individuals. Metabolite profiles diverged between Bd-infected moribund frogs and both uninfected control and sub-clinically infected groups in both skin and liver tissues. Several key metabolites discriminated populations and exposure groups including markers of sugar metabolism in the liver profiles, and within infected skin tissue, a number of biogenic amines correlated with disease progression. The confirmed identification of key metabolites was supported by accurate mass quadrupole time-of-flight/MS analysis. Differences between populations were less pronounced, with pantothenic acid being found consistently across analyses as significantly differentially abundant within skin samples. This is the first metabolomics investigation on the skin and liver association of the pathophysiology of chytridiomycosis.

POSTER 195

Marine metabolomics: the effect of environmental and biotic interaction on the metabolome of seagrasses

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Environmental metabolomics has become interesting in marine ecological studies. One example is the revealing of new insights in response, adaptation and tolerance mechanisms of the seagrass Zostera marina to environmental stress, invasive species and climate change. Remarkably the underlying molecular mechanisms to this global threats are largely unknown and have not been experimentally tested so far by metabolomic approaches. However this is essential to understand the future of seagrass ecosystems - functioning as key marine ecosystems in terms of carbon dioxide sequestration, nursery for juvenile and therefore proving the foundation for fishery industry, primary food source and preventing sediment erosion. Here we present an marine ecological metabolomics approach to understand molecular responses towards future warmer climate scenarios. To assess adaptation and response mechanisms of seagrasses we exposed Z. marina to a diurnal light/dark cycle under anoxia and assessed the metabolic response by measuring the metabolome with gas chromatography coupled to

mass spectrometry (GC–TOF-MS). In a second experiment we assessed the effect of species interaction (mussels) and environmental factors (light) on seagrasses by metabolic profiling of Z. marina by GC-qTOF-MS and LC-qTOF-MS under 5 different analytical conditions: (1) RPOSTER LC-MS ESI+; (2) RPOSTER LC-MS ESI−; (3) HILIC-MS ESI+; (4) HILIC-MS ESI−; and (5) GC-MS. We used uni (ANOVA) and multivariate (PCA and PLS-DA) statistics to illustrate the effects of environmental influences on the metabolome of the seagrass Z. marina and integrated this results with traditional ecological measurements. During anoxia and light exposure the roots showed an altered metabolome whereas the leaves were only marginally affected, indicating that photosynthetically derived oxygen could satisfy the oxygen demand in the leaves but not in the roots. Nocturnal anoxia caused a biphasic shift in the metabolome of roots and leaves. The first phase, after 15 h under anoxia and 3 h of darkness showed a fast increase of lactate, pyruvate, GABA, succinate, alanine and a decrease in glutamate and glutamine. The second phase, after 21 h under anoxia and 9 h of darkness showed a decrease in lactate and pyruvate and an increase in alanine, GABA and succinate. This reprogramming of the metabolome after 21 h under anoxia indicates a possible mitigation mechanism to avoid the toxic effects of anoxia. A pathway enrichment analysis proposes the alanine shunt, the GABA shunt and the 2-oxoglutarate shunt as such mitigation mechanisms that alleviate pyruvate levels and lead to carbon and nitrogen storage during anoxia. We demonstrates the applicability of metabolomics to assess low oxygen stress responses of Z. marina and allows us to propose an anoxia recovery model. In a second experiments varying light exposure and species interaction of Z. marina with blue mussels were reflected in different metabolic-profiles of all sample groups in all analytical conditions. Multivariate data-analysis (PCA/PLS-DA) indicated light exposure as main variance (component1=30-45%) between sample groups whereas mussel presence explained 7-13% (component 2 and 3) of the variation in sample grouping. Light modified the effect of mussels on the metabolic profiles indicated through separation of the sample groups on different principal components and thus by different metabolites. Traditional ecological and biogeochemical could resolve effects of light but not of the mussel treatment. Remarkably integration with metabolomics data revealed community respiration and sulfide pressure as driving factors in this experiment. We integrated multiplatform metabolomics data with traditional ecological and biogeochemical measures to reveal underlying molecular mechanisms of ecological phenomena.

POSTER 196

Metabolic Alterations by Dose-Dependent Gamma Irradiation in Human Fibroblasts

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Radiation exposure is a threat to public health because it causes many diseases, such as birth defects and cancers, due to genetic modification of cells. Compared with the past, a greater number of people are more frequently exposed to higher levels of radioactivity today, not least due to the increased use of diagnostic and therapeutic radiation-emitting devices. In this study, UPLC-QTOF-MS-based metabolic profiling was used to investigate radiation-induced metabolic changes in human fibroblasts. After exposure to 1 and 5 Gy of gamma-radiation, the irradiated human primary cultured dermal fibroblasts were harvested at 24, 48, and 72 h and subjected to global metabolite profiling analysis. Mass spectral peaks of cell extracts were analyzed by pattern recognition using principal component analysis (PCA) and partial least squares-discriminant analysis (PLS-DA). The cells irradiated with 1 Gy returned to control levels at 72 h post radiation, whereas cells irradiated with 5 Gy were quite unlike the controls; therefore, cells irradiated with 1 Gy had recovered, whereas those irradiated with 5 Gy had not. Lipid and amino acid levels increased after the higher-level radiation, indicating degradation of membranes and proteins. We suggest that MS-based metabolite profiling of gamma-radiation-exposed human cells provides insight into the global metabolic alterations in these cells.

POSTER 198

Metabolomics Reveals that Aryl Hydrocarbon Receptor Activation by TCDF Impacts Gut Microbiome-Host Inflammation and Metabolic Homeostasis

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Environmental exposure to dioxins and dioxin-like compounds poses a significant health risk for human health. Developing a better understanding of the mechanisms of toxicity through activation of the aryl hydrocarbon receptor (AHR) is likely to improve the reliability of risk assessment. In this study, wild type, Ahr-/- and gnotobiotic male C57BL/J6 mice (6-week-old, 5-6 per group) were treated with and without TCDF for five days at 24 μg/kg body weight. Combined 16S rRNA metagenomics, 1H nuclear magnetic resonance (NMR) metabolomics, LC/GC-MS metabolite profiling, and biochemical assays were used. Weighted UniFrac principal coordinate analyses of 16S rRNA sequencing results indicated that TCDF exposure induced a remarkable change in the overall gut microbiome population whereas no significant changes observed between TCDF-treated Ahr-/- mice and vehicle-treated Ahr-/- mice. Firmicutes and Bacteroidetes exhibited significant changes with reduction of Firmicutes/Bacteroidetes ratio after TCDF exposure. Furthermore, TCDF-treated mouse cecal contents were enriched with genus Butyrivibrio and Flavobacteria, but depleted in the genus Clostridia and Oscillobacter in comparison with the vehicle-treated mice. Interestingly, we found significant elevation of inflammatory factors in serum including G-CSF, Eotaxin, IPOSTER 10, MCPOSTER 1, Il-6 and Il-12 in TCDF-treated gnotobiotic mice compared to vehicle-treated gnotobiotic mice whereas no significant differences were observed between vehicle-treated and TCDF-treated conventional mice. These observation suggested the microbiome protected inflammation induced by TCDF. 1H NMR-based metabolomics results showed that TCDF led to hepatic lipogenesis and inhibition of gluconeogenesis and glycogenolysis in wild type mice and these changes were AHR-dependent. Further, significant elevation of hepatic glutathione, glucose and glycogen but reduction of lactate and amino acids was observed in TCDF-treated gnotobiotic mice suggesting oxidative stress and inhibition of glycolysis induced by TCDF. These findings provide new evidence that exposure to persistent environmental contaminants strongly impacts the gut microbiome, host inflammation and metabolome.

POSTER 199

1H NMR-Based Metabolomics and GC-MS Metabolite Profiling Reveals the Impact of Tempol on the Host-Gut Microbiota Metabolic Pathway

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Microbes throughout the gastrointestinal tract interact with and signal the host through the farnesoid X receptor (FXR) to modulate metabolic pathways including bile acid metabolism. Tempol, a stable free radical nitroxide, is known to cause weight loss, improves many obesity-related complications such as non-alcoholic fatty liver disease, and has recently been found to exert these salubrious effects through modulation of the gut microbiota and indirectly antagonizing intestinal FXR activity. However, how tempol treatment impacts the gut microbiota remains unclear. To investigate the metabolic changes associated with tempol treatment, mice were administered tempol by gavage for five days. Metabolites from mouse cecal contents, feces, and liver were analyzed by a combination of GC-MS (Agilent 5975 GC/MSD) and

1H NMR (Bruker Avance-III 600 MHz). Additionally, 16S rRNA gene sequencing and metagenomics were used to determine how tempol impacted the gut microbiota both in terms of population as well as metabolic potential. A dose-dependent decrease in bacterial fermentation was found in tempol-treated mice indicated by significantly lower level of SCFAs (acetate, butyrate, and propionate) in cecal and fecal extracts as well as increased excretion of fecal oligosaccharides. Bomb calorimetry of fecal pellets confirmed these results finding that tempol-treated mice had greater energy excretion compared to vehicle-treated mice. Moreover, 1H NMR analysis of liver extracts showed decreased sugar and amino acid storage and increased uridine, phenylalanine, and tyrosine in tempol-treated mice indicating an upregulation of glucogenic and ketogenic activity and increased glycolysis. The 16S rRNA gene sequencing data implicated Lactobacillus spp. as a potential target of tempol and the metagenomics data was useful for confirming the metabolomics and metabolite profiling data. Tempol treatment decreases energy availability by inhibiting microbial fermentation and causes a compensatory shift in host liver metabolism.

POSTER 200

NMR and LC-MS based Serum Metabolomics and Lipidomics profiling of Hemochromatosis in Sumatran Rhinoceros (Dicerorhinus sumatrensis)

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A variety of wildlife species maintained in captivity are susceptible to hemochromatosis, a disease resulting from the deposition of excess body iron into insoluble iron clusters in soft tissue. The Sumatran rhinoceros (Dicerorhinus sumatrensis) is one of the rhinoceros species which has adapted to a low-iron diet and is susceptible to iron overload. Hemosiderosis increases with time in captivity, but the underlying cause and reasons for differences in susceptibility to hemochromatosis within the taxon remains unclear. To investigate the metabolic changes during the development of hemochromatosis and potentially increase our understanding of susceptibility differences, the metabolome and lipidome of serum from a Sumatran rhinoceros that had died from liver failure caused by hemochromatosis were investigated by NMR and LC-MS. Serum samples from a female Sumatran rhinoceros at the Cincinnati Zoo were collected periodically throughout the year from 2006 to 2014. In this preliminary analysis, 15 samples were categorized into three groups (n=5 each): 1) healthy- 4-7 years before diagnosis, 2) pre-illness- 1-3 years before diagnosis, and 3) illness- after diagnosis based on clinical symptoms. The samples were extracted using a modified Bligh and Dyer extraction method. The top aqueous layers containing polar metabolites were further divided and analyzed by 600MHz NMR and UPLC-QTOF-MS. The bottom organic layers containing lipids were analyzed in UPLC-QTOF-MS using positive and negative ion modes. Multivariate and univariate analyses were performed to identify changes in the serum metabolome and lipidome during disease development and progression. PCA analysis revealed differences between healthy and illness samples, and close similarity between healthy and pre-illness samples in NMR and MS data of the serum metabolome and lipidome. A total of 25 altered metabolites were identified by univariate SDS analysis of binned NMR spectra comparing combined healthy and pre-illness groups with illness groups. Altered metabolites between groups were identified based on ANOVA (Poster value <10-2) and OPLS-DA analyses of MS data. Significant metabolites found in MS and NMR data were taken together to determine the biological significance in this study. The metabolic signatures for weight loss, induced fat and muscle breakdown, were indicated by the detection of 12 altered amino acids and an increase in triglycerides. Impaired fatty acid and glycerophospholipid metabolism was indicated from altered lipid levels (e.g.phosphatidylcholine, phosphatidylethanolamine, phosphatidylserine, linoleic acid and its intermediates). Changes in bile acid synthesis during the disease progression was indicated by the increased bile acid concentrations, including cholic acid, cholanic acid, glycocholic acid, and taurodeoxycholic acid (bile salt). Increases in choline and triglyceride coupled with consistent decreases in choline-containing compounds (phosphocholine, phosphatidylcholine, and LysoPC) suggested an altered bile secretion. Additionally, an increase in the production of ferritin, the

main intracellular iron storage protein, was strongly indicated by identification of altered metabolites found in porphyrin metabolism. These metabolites included protoporphyrinogen IX, coproporphyrinogen I/III, and dehydroisocoproporphyrinogen, all of which were observed to increase as early as 1 year prior to diagnosis (pre-illness). This finding may explain observed decreases in branched-chain amino acids and glycine, since these amino acids are highly involved in ferritin production. Taken together, current analysis suggests significant alterations in fatty acids, glycerophospholipids, porphyrin, protein, and bile acid metabolism during hemochromatosis progression. Time-series analysis monitoring metabolic changes during disease progression may lead to a development of new diagnostic methods for hemochromatosis.

POSTER 201

Metabolomics identifies a biological response to chronic low-dose natural uranium contamination in urine samples

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Because uranium is a natural element present in the earth's crust, the population may be chronically exposed to low doses of it through drinking water. Biological effect of such contamination has been scarcely investigated so far, but few recent studies reported molecular changes in some major metabolic pathways including intestinal inflammatory pathways, the reproductive system, brain pro- and anti-oxidant activity and xenobiotic metabolism. The aim of this study was to use a more sensitive approach, metabolomics, to assess the biological changes in rats caused by ingestion of natural uranium (natU) in drinking water for 9 months, and to identify potential biomarkers related to such internal contamination. We used 2x10 Sprague-Dawley rats; contaminated animals drank water at a final natU concentration of 40 mg/L (2.7 mg/kg of rat), being 3 times as much as the highest uranium concentration found naturally in Finnish water wells. Clinical and biochemical tests included food/water intakes and diuresis measurements, whole body and kidney weighting, blood cell count, and markers assays for kidney, liver and cardiovascular functions (proteins, lipids, enzymes, ions). For metabolomics analyses, urine samples were diluted to a quarter and analyzed on a HPLC-ESI-QTOF; raw data were deconvoluted with XCMS using the CAMERA package, and filtered using an in-house R script; statistical analyses were performed either with SIMCA-P+ (PCA, PLS-DA) or R (ROC curves and AUC). We observed no pathology and standard clinical/biochemical tests were unable to discriminate control from contaminated rats, conversely to LC-MS metabolomics. The PLS-DA performed on the 1,376 features detected in urine samples (after filtration) resulted in a robust and validated discrimination between the two groups of animals (R2Y=92%, Q2Y=55%, CV-ANOVA=0.009). The 95 most discriminating variables, selected according to their VIP score (above 1.8), were used for a PCA. The first principal component described 40% of total variance and represented the inter-group variation, while the second principal component described 14% of total variation and represented the inter-individual variability. A new PLS-DA performed on these 95 most discriminating variables resulted in a more robust and validated discrimination (R2Y=89%, Q2Y=74%, CV-ANOVA<0.0005). The top 40 features exhibited a quite satisfactory discriminating power, as their AUC under the ROC curve was above 71% (above 80% for the top 27). After identification, the most discriminating metabolites appeared to be involved in tryptophan and nicotinate/nicotinamide pathways. In particular, N1-methylnicotinamide, which level was 7 times lower in control rats, had the greatest

discriminating power (AUC=100%). These novel results establish a proof of principle for using the metabolomics approach to address the in vivo impact of a chronic low-dose natural uranium contamination. They open interesting perspectives for understanding the underlying biological mechanisms and designing a dianostic test in the field of low-dose radiotoxicology [1]. [1] Metabolomics identifies a biological response to chronic low-dose natural uranium contamination in urine samples. Grison S et al., Metabolomics 2013, 9(6):1168-1180. The metabolomics approach is appropriate to address the in vivo impact of a chronic low-dose natural uranium contamination.

POSTER 202

A smartphone metabolomics platform and its application to the assessment of cisplatin-induced kidney toxicity

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In the world, access to healthcare is limited due to insufficient medical infrastructure or limited availability of medical devices. The application of smartphones to medical devices has been gaining attention in addressing accessibility and cost issues in healthcare, and has been demonstrated using customized smartphone hardware and/or software. Metabolomics has also spawned many medical applications but requires highly sophisticated and expensive equipments. Given the developments in both smartphones and metabolomics, a smartphone-implemented general-purpose metabolomics platform seems tantalizingly on the verge of opening a new door to affordable healthcare devices. In this study, we propose an integra ted metabolomics approach that bundles all of the essential metabolomics functions, including data acquisition, processing, statistical analysis, visualization and prediction, in a smartphone. Cisplatin or saline were administered to rats. Urine samples were collected over the course of 24h before and after the injection. Standard UV–vis spectroscopy was performed on the urine samples and the spectra were obtained for the 400–700 nm wavelength range. The paper spectroscopy kit was attached to the smartphone and the same urine samples were used for the smartphone data acquisition and spectra were acquired by the built-in smartphone camera. Each photo was imported to the LearnLight spectrometry application and converted to a text file containing wavelength and intensities. The text was imported to the Addi platform for Android and multivariate statistical analysis was performed, then results were visualized with with in-house written script for Addi plot. We set up an animal model for cisplatin toxicity. Blood and kidney samples obtained from animals administered saline (control) or 10 cisplatin (cisplatin) were analyzed. Both BUN and Cr were significantly higher in the cisplatin group, and tissues from the cisplatin group exhibited an irregular tubular and cellular morphology thus suggesting kidney toxicity. Before investing any major effort in smartphone metabolomics, we evaluated the feasibility of utilization of visible light by testing the capability of light-absorption-spectroscopy-based metabolomics in differentiating animal toxicity groups. PLS-DA multivariate model built from the spectral data well differentiated the control from the cisplatin urine samples, and the method correctly predicted the toxicity group of all 8 test samples. These results suggest that visible light is a viable option for the metabolomics approach. A connector case for spectrometer and smartphone built with acryl plates and attached to a commercial smartphone case, and an educational paper spectrometer kit employing diffraction grating film was used. With the complete smartphone spectrometer assembly, the visible spectra of rat urine samples were obtained. The intensity values of the spectra, and their sum were extracted using the LearnLight application and exported as text files. These data were analyzed with the PLS-DA algorithm implemented in the Addi mathematic platform and visualized with the Addi-plot applications. The score plot with the first two PLS components showed a good distinction between control and cisplatin groups. The actual performance of the differentiation model was tested with the same algorithm as used in the UV–vis spectrometer case. Out of the 8 test samples, 7 samples were correctly predicted. We built the metabolomics platform with cost, portability and ease of use in mind. We also completed a proof-of-concept wherein the metabolomics platform was successfully used to assess cisplatin toxicity in a rat model. We provide a proof-of-concept of smartphone metabolomics platform applying to the assessment of cisplatin-induced kidney toxicity in a rat model.

POSTER 203

Mass spectrometry-based lipidomics to study early event of naphthalene toxicity in mice

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Naphthalene is the simplest polycyclic aromatic hydrocarbon and widely exists in the environment. Previous studies have revealed that naphthalene-induced airway non-ciliated (Clara) cell injuries in mice at earlier time points. Since the changes of cell morphology may be greatly related to membrane components, it is important to understand toxic mechanisms in the development of naphthalene toxicity by lipidomic approach. Currently, we focus on the most abundant membrane lipids, phosphatidylcholines. Male ICR mice were treated with 200 mg/kg naphthalene by intraperitoneally (i.p.) for 1, 2, 3 or 6 hours. H &E stain and immunohistochemistry were used to characterize cellular morphological changes in the lung. Lung tissues and serum were also collected and prepared for mass spectrometry (MS) based lipidomic approach. Phosphatidylcholines were analyzed by ultra performance liquid chromatography coupled with tandem mass spectrometer (UPLC-MS/MS). After pre-processing MS data, multivariate statistical analysis, such as partial least squared discriminate analysis were used to analyze phosphatidylcholine variation among samples. The histopathologic results showed Clara cells were injured as early as 2 hours post dose. As time period increased, Clara cell injuries became more severe. Hundreds of phosphatidylcholine species were identified. Phosphatidylcholine profiles from the lung tissues of mice treated with naphthalene could be discriminated from the control group in partial least squared discriminate analysis model. Moreover, a trend of time series was identified. Numerous phosphatidylcholines were associated with naphthalene toxicity. Changes of specific phosphatidylcholine species can explain the development of naphthalene-induced airway injury.

POSTER 204

Metabolic Phenotyping reveals a Lipid-mediated Proinflammatory Response to Ionizing Radiation

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Exposure to ionizing radiation has dramatically increased in modern society, raising serious health concerns. The deleterious consequences of ionizing radiation exposure can result in cancer and non-cancer-related diseases, including cardiovascular diseases and cognitive decline. The molecular response to ionizing radiation, however, is still not completely understood. The capability to phenotype blood provides novel opportunities to investigate the molecular response to radiation, thus yielding mechanistic insights as well as discovering novel biomarkers. Here we applied a novel multi-platform, mass-spectrometry-based metabolic phenotyping strategy to investigate in mouse serum the molecular response to acute, gamma-radiation exposure, and describe a molecular biosignature that has the potential to be used as an indicator of radiation exposure and as a potential target for therapeutic interventions. Mice were irradiated or subjected to the same treatment minus irradiation. Blood was collected and serum samples prepared. Before extraction, a mixture of internal standards was added to the serum. Polar metabolites were separated using an UPLC system fitted with a BEH HILIC 2.1x100 mm, 1.7-µm column. Total lipid analysis was performed on a microfluidic device with a C18 150 µm x 100 mm, 1.7-µm column. Global metabolic profiling was performed on hybrid Q-TOF systems. Initial discovery data were further investigated using complementary, multiplexed, metabolic-profiling approaches. Targeted analyses were performed on triple quadrupole MS systems. The results were integrated for the generation of a unique metabolic biosignature related to radiation exposure. A global, molecular profiling revealed that mouse serum undergoes a series of significant molecular alterations following radiation exposure. We identified and quantified bioactive metabolites belonging to key biochemical pathways and low-abundance, oxygenated, polyunsaturated, fatty acids (PUFAs). Exposure to gamma radiation induced

a significant increase in the serum levels of ether phosphatidylcholines (PCs) while decreasing the levels of diacyl PCs carrying PUFAs. In exposed mice, levels of pro-inflammatory, oxygenated metabolites of arachidonic acid increased, whereas levels of anti-inflammatory metabolites of omega-3 PUFAs decreased. Our results indicate a specific serum lipidomic biosignature, which could be utilized as an indicator of radiation exposure and as novel target for therapeutic intervention. If validated in humans, monitoring such a molecular response to radiation exposure might have implications not only for radiation pathology but also for countermeasures and personalized medicine. Metabolic phenotyping revealed an inflammatory component in the molecular response to ionizing radiation.

POSTER 205

Local and systemic effects of exposure to biodiesel exhaust on humans detected by global metabolomics

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Biodiesel is a renewable fuel mainly consisting of esters, whereas diesel is mainly paraffinic and aromatic. With both biodiesel and diesel engines, energy is released in a series of small combustion as fuel reacts chemically with oxygen from the air. Combustion produces particulate matters which can enter human body and cause damage. Replacing diesel with biodiesel may have beneficial effects in ecology; however, public health effects are still unknown. In this project our goal was to study the effect of exposure to biodiesel exhaust in a controlled setting and compare with ambient air exposure. 15 non-smoking healthy males were exposed to ambient air and biodiesel exhaust for one hour in a randomized order. Peripheral blood was sampled at four time points: pre-exposure as well as two, six and 24 hours post-exposure. Bronchoscopy samples including 2x20 ml bronchial wash (BW) and 3x60 ml bronchoalveolar lavage (BAL) were collected six hours post exposure. A methanol/water based protocol was used on the plasma samples for extraction of low molecular compounds (<900 Da). This was followed by derivatization and analysis using gas chromatography coupled to time of flight mass spectrometry (GC-TOFMS). Using in-house script, data were processed which enabled identification and quantification of 76 metabolites. Furthermore, a newly developed methanol/water based protocol was used for extraction and identification of low molecular compounds in BW and BAL samples, resulting in identification of 65 metabolites. Univariate, ANOVA and multivariate analysis were used to examine the data and highlight molecular profiles characteristic for each type of exposure and over time. Preliminary results showed differences in local and systemic responses in metabolite profiles after biodiesel exhaust exposure compared to ambient air exposure. Investigating biodiesel exhaust exposure effects on human plasma and lung compartment metabolome profiles using randomized controlled trial followed by Metabolomics.

POSTER 206

Metabolic Changes in Plasma of Rats with Liver Injury Induced by Carbon Tetrachloride in Combination with Thyroxine and Reserpine

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Carbon tetrachloride (CCl4) is commonly used as a toxicant to induce liver injuries. However, clinical manifestations of patients with liver disease are far more complicated. Besides liver dysfunction, a part of chronic hepatitis B patients, for example, are often accompanied with asthenia, fever, depression, and erectile dysfunction for male and menoxenia for female, that is known as “liver and kidney Yin deficiency” in terms of traditional Chinese medicine. Therefore, in this study, we established a novel liver injury model by using a combination of thyroxine, reserpine and CCl4 to imitate liver disease patients with those symptoms. UPLC−MS based plasma metabolomics was performed to elucidate the pathological status and the adaptive response in vivo caused by chemical stimuli. Wistar rats were randomly divided into 3 groups: control, model I and model II. The rats in model II group were administrated with thyroxine (160 mg/kg) and reserpine (1 mg/kg) for 10 consecutive days, control and model I were administrated with distilled water. On day 11, two model groups were received CCl4 (0.5 mL/kg) in corn oil, and control were received corn oil only. Blood was collected just before (0 h) and 2, 6 h after CCl4 administration. Time-course metabolic changes in plasma of rats were investigated by means of UPLC−MS. A combined statistical strategy of 2-way ANOVA and multivariate analyses was applied to find the potential biomarkers associated with the pathological process induced by the combinational drugs. Blood at 6 h after CCl4 administration was used for biochemical indicators test. CCl4 caused rats no matter in model I or model II significant elevations of plasma ALT, AST, LDH, TBIL, DBIL and CHE accompanied with decreases for ALB levels. Those indicators of rats in model II changed more dramatically than in model I. The significant level increases for plasma AST and ALT confirmed the hepatic injuries observed in histopathology. A PLS-DA model was performed to obtain an overview of systemic alterations in metabolome of rats in model II and control. PLS-DA score plot showed that the two groups clustered closely before CCl4 administration and gradually separated from each other after CCl4 were received for 2 or 6 h. Both of the groups moved away from the original spot, but model II group moved a longer distance and faster than the control group. It indicated metabolome of the rats in model II had changed more significantly due to exposure to CCl4. We also got a similar result when comparing model I with control. Sixteen and 34 metabolites were identified to distinguish between the two model groups and control respectively according to their VIP values and locations on s-plot based on OPLS-DA model. Two-way ANOVA test were employed to recognize metabolites with significant changes between sample groups produced by vehicle, CCl4, or their interaction. As a result, a total of 10 metabolites were picked out as potential biomarkers related to pathology of liver injury for model I, and 20 metabolites for model II. From the result of pathway enrichment analysis, three pathways were disturbed in model I, including valine and leucine biosynthesis, histidine metabolism and glycerophospholipid metabolism. Two more pathways, porphyrin and chlorophyll metabolism and pyrimidine metabolism, were disturbed in model II. Metabolomics strategy was applied to evaluate a novel rat model of liver injury accompanied with common symptoms of liver disease.

POSTER 207

Metabolomics approach to evaluate fate and impact of natural herbicides in the environment

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The increasing use of natural products of botanical origin (botanicals) in the agriculture practices has led to increasing concern about the environmental fate of these complex substances due to the lack of ecosafety data with regulatory authorities. A potential approach would be to define suitable protocols to analyse the impact and fate of botanicals in the environment. These substances are assumed to be biodegradable, which implies that the degradation pattern of these substances and impact of these xenobiotics and their byproducts on soil metabolome can be revealed using metabolomics profiling. We here outline a novel approach to agrochemicals risk assessment that is based upon non-targeted metabolomics study of herbicide contaminated and non-contaminated soil A soil free of any previous herbicide exposure was selected for the laboratory microcosm experiment with synthetic (sulcotrione) and

natural (leptospermone) β-triketone herbicides following OECD guidelines. For each time and treatment condition, five soil samples (10.0 g each) were treated in plastic boxes with a methanolic solution of sulcotrione or leptospermone corresponding to the recommended application dose for each triketone. Microcosms were maintained for 90 days and samples were taken at different time points for the extraction of soil metabolites in ethyl acetate. A metabolomic platform, which integrates a LCMS-based metabolic fingerprinting, multivariate analysis (including PCA, PLSDA, MANOVA) has been applied in order to characterize the changes in the soil metabolome over treatment and time in a laboratory microcosm condition. A common extraction system was validated for the efficient extraction of test compounds from the soil samples. Degradation pattern of test herbicides was also monitored by HPLC to compare with LCMS-based approach. Under our conditions, the disappearance of both sulcotrione (conventional herbicide) and leptospermone (natural herbicide) has been observed which suggest the resilience of soil metabolome over days after treatment. PCA analysis indicated the optimal view of the separation between the controls and the treated samples. The PCA modelling applied to LCMS-based metabolic fingerprints allowed to recognize the effects due to treatment and time. It is noteworthy that early resilience of soil metabolome was observed after leptospermone treatment (day 15) compared to sulcotrione (day 45) even with 5-fold higher dose. Variables important in projection (VIP) are being characterized at time points with maximum variability. Knowledge about characteristic compound could explain the difference in soil metabolome after treatment and fate of triketone herbicides in the selected soil types. The results of this preliminary study performed on pure compounds invites us to test this non-targeted metabolomics approach to analyze environmental fate and impact of plant crude extracts (mix of natural substances) used for crop protection. This approach can be applied to define suitable protocols to analyze fate and impact of botanicals in the environment.

POSTER 208

Using Targeted Metabolomics to Measure Multi-Organism Responses to Great Lakes Sediment, Effluent and Surface Water Exposures

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The Great Lakes Basin in Southern Ontario receives a variety of contaminants including metals, pesticides, pharmaceuticals, personal care products and other endocrine disrupting compounds from multiple point sources including municipal wastewater treatment plants. The sheer number and diversity of chemicals makes monitoring of the exposome expensive and non-comprehensive. Effects-based monitoring can, based on the measurement of multi-omic responses in sensitive and sentinel organisms, potentially identify sources more likely to cause ecosystem harm, and serve as early warning systems. Quantitative metabolite concentrations of conserved systems facilitate cross-species translation. In the present work, a suite of metabolites was measured in fathead minnow, rainbow trout and hexagenia larvae following laboratory exposures to effluent, sediment and surface water, in order to quantify exposure effects. Wastewater treatment plant effluent (WWTP) from two sites (Woodward and Dundas) in Hamilton harbour, surface water from Lake Ontario and Lake Erie, and Hamilton harbour sediment were sampled and used for exposure studies. Rainbow trout, juvenile fathead minnow and hexagenia larvae were exposed for 48 h ± 2 (n = 10 replicates). Concentrations of a set of 217 metabolites including amino acids and biogenic amines, ∑hexoses, fatty acids, bile acids, acylcarnitines, sphingomyelins, and glycerophospholipids were measured in the samples using a targeted and quantitative LC-MS/MS and FI-MS/MS platform. A suite of multivariate statistical techniques were used to identify metabolites varying between treatment and controls and to correlate metabolite changes with available water quality and contaminant concentration data. Preliminary metabolomics data for rainbow trout liver and whole hexagenia larvae indicate distinct patterns of metabolite changes between the different exposure studies. Rainbow trout exposed to effluent from the Woodward WWTP showed significant changes in over 40 of the approximately 200 metabolites quantified, including a number of amino acids and biogenic amines. Surface water and effluent from the Dundas WWTP resulted in fewer metabolites significantly changed. These differences were associated with higher contaminant levels measured in the Woodward WWTP effluent. Preliminary metabolite measurements in hexagenia larvae exposed to the

same effluent and surface water samples show similar patterns with surface water exposures resulting in few metabolite changes, and those exposed to the WWTP effluents showing more significant changes in metabolite concentrations. A number of the same metabolites show changes both in the fathead minnow and hexagenia larvae, indicating potential to study translation of response across species. Data from sediment exposures are under analysis and will be presented and related to aqueous responses. These metabolite measurements are part of a larger study comprising transcriptomic measurements of the same samples, and correlations of the changes with available water quality and exposome concentration data. Targeted metabolomics in sentinel species exposed to complex environmental matrices generate translatable metabolite concentration data to support effects-directed analysis.

POSTER 209

Cell-based metabolomics for assessing chemical exposure and toxicity of environmental surface waters

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Waste water treatment plants (WWTPs), concentrated animal feeding operations (CAFOs), mining activities, and agricultural operations release contaminants that negatively affect surface water quality. Traditional methods using live animals (e.g. fish) to monitor/assess contaminant exposure and impacts in affected ecosystems are both resource and time intensive and are not sustainable in the long term. This study describes an optimized cell culture-based method employing metabolomics combined with high throughput NMR spectroscopic analysis to obtain multi-endpoint cellular responses to stressor exposure. We report results of applying cell culture-based metabolomics using Zebrafish (Danio rerio) liver cells (ZFL) to determine impacted biochemical processes and putative biomarkers of exposure to surface waters from contaminated and pristine sites. Several surface waters were collected at a Great Lakes Area of Concern (AOC), specifically the St. Louis River AOC into which the Western Lake Superior Sanitary District WWTP flows. ZFL cells were then exposed to culture media prepared with the surface water samples using different doses and durations as well as media prepared from water collected at a reference site and using purified laboratory water. The polar fractions of cellular extracts were analyzed by flow NMR automation. Cellular responses were determined by evaluating changes in endogenous metabolite profiles using a combination of PCA and t-test filtered difference spectra. PCA analysis indicated that metabolic changes were time- and dose-dependent for the ZFL cells exposed to the environmental surface waters collected at two WWTPs and downstream sites. However, no significant changes were observed after exposure to the surface water from the pristine site, compared to the control water (Lake Superior water). The t-test filtered difference spectra determined that metabolites involved in energy production varied as a function of the exposure concentration and duration. The other primary responses observed were impacts on lipid and glutathione metabolism. Cell-based metabolomics is applied to field studies.

POSTER 210

NMR-based Metabolomic study of brain early exposed to PCB-NDL in mice

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The rapid growth of anthropogenic activities has led to the use and wasting of numerous chemical compounds in the environment, exposure to which may constitute a threat for animal and human well-being and health. In particular, recent epidemiological studies have shown an increase of neurological and mental pathologies in child. Environmental pollutants are strongly suspected to induce these diseases when exposure occurs during early life stages (developmental period). The aim of this work was to study in mice the impact of a perinatal exposure (in utero and during lactation) to PolyChloroBiphenyls (PCBs) on the plasmatic and cerebral metabolism of the offspring by using a global approach: proton NMR-based metabonomics. Pregnant mice have been exposed to PCBs either by force-feeding a mixture of 6 standard Non-Dioxin-Like PCBs (NDL-PCBs, two different concentrations), or via a naturally contaminated food matrix (eel, two different contamination levels). 15 days after birth, male and female young mice were sacrificed and plasma and brain (cortex and cerebellum) have been sampled. 600 MHz proton NMR analyses have been performed on plasma samples, and on both aqueous and lipidic extracts of brain (cerebellum and cortex). Multivariate statistical analyses (Partial Least Squares-Discriminant Analysis) of NMR data allow the separation of the different groups according to the exposure levels and sources. Specific metabolic fingerprints reflect the effects of the exposure to NDL-PCBs. Results also indicate that metabolic fingerprints obtained from male and female are different, showing that the impact of the exposure to NDL-PCBs during pregnancy and lactation is different depending on the sex of the offspring. Variations in various amino acids known as brain neurotransmitters (glutamate, glycine, GABA) and energy-related metabolites (lactate, creatine) have been observed in brain of mice exposed to PCBs. Numerous metabolic pathways were shown to be disrupted, among which the glutamatergic pathway may represent a key point for susceptibility of the brain to NDL-PCBs. Metabolomics is a powerful tool to assess the neurotoxicity of early exposure to environmental contaminants (low doses and in mixture).

POSTER 211

A targeted metabolomics pipeline for elucidating imidacloprid sublethal toxicity in the freshwater snail Lymnaea Stagnalis central nervous system

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Neonicotinoids pesticides received significant attention because of the possible connection between their use and the decline of honeybee colonies. Recently, more evidences are suggesting that the widespread use of this class of pesticides is harmful to non-target species as well. Imidacloprid is one of most employed neonicotinoids and is an environmental pollutant of concern. Imidacloprid acts as agonist on the insects nicotinic acetylcholine receptors (nAChRs) but recent reports indicate that non-target species are also affected. Therefore, the aim of this study is to elucidate imidacloprid-induced sublethal effects by a targeted metabolomics strategy in the central nervous system (CNS) of the non-target species, the freshwater snail Lymnaea stagnalis. A ten days exposure to imidacloprid environmental relevant concentrations (0.1 and 1 µg/L) and higher concentrations (10 and 100 µg/L) was executed. Effects on reproduction and acetylcholine esterase (AchE) were evaluated. A metabolomics targeted approach based on HILIC-ESI-QqQ was employed to perform neurotransmitters profiling. The hydrophilic/hydrophobic fractions were analysed respectively by HILIC-ESI-ToF and GC-APCI-ToF. Data analysis was performed by screening the HR-MS chromatograms for metabolomics standards using PathwayScreener and the MSMLS library (IROA Technologies), consisting of more than 600 metabolites. Multiple t-test with FDR correction were employed to determine the metabolites contributing to the difference between control group and exposed groups. In order to provide insights in the metabolic pathways involved with imidacloprid toxicity, biochemical networks were created. A targeted metabolomics approach based on neurotransmitters profiling and the screening of the comprehensive metabolite library enabled to reveal significant changes in hydrophobic and hydrophilic metabolites in the CNS of L. stagnalis exposed to environmental relevant concentrations of imidacloprid. Indeed, significant changes were found in many amino acids, fatty acids and neurotransmitters. The biochemical network mapping of these quantitative results helped to better identify the connections between the significantly

changed metabolites, and therefore the pathways potentially affected. Metabolites that showed a decrease/increase in accordance to the increasing exposure concentrations and therefore, potential biomarkers of imidacloprid exposure are carnitine, betaine, choline, GABA, phenylalanine, tryptophan and serotonin. An UPoster regulation was observed for carnitine, betaine, phenylalanine, choline and GABA, whereas a down-regulation was shown for tryptophan and serotonin. Regarding the non-polar metabolites, elaidic acid, oleic acid, heptadecanoic acid and stearic acid were down-regulated due to imidacloprid exposure. In this study, the involvement of different metabolic and neuronal pathways was highlighted in a non-target species. In addition, potential biomarkers of exposure have been discovered for sublethal exposure of imidacloprid in the freshwater snail L. Stagnalis. The results of this study clearly indicate that significant changes at the molecular level are induced by much lower imidacloprid concentrations than those inducing significant phenotypical effects. As a matter of fact, traditional toxicity effect parameters were compared to metabolomics and they resulted to be less effective in showing effects of imidacloprid exposure in the CNS of L. stagnalis. Targeted data analysis workflow facilitates the extraction of biologically relevant information in environmental metabolomics.

POSTER 212

NMR Metabolomics for the Evaluation of Diahrretic Shelfish Poisoning Toxin Effects

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Diarrheic shellfish poisoning (DSP) is a severe human illness caused by the consumption of seafood contaminated with lipophilic toxins produced by marine dinoflagellates. Okadaic acid is the lead DSP toxin, but an increasing number of related compounds have been isolated and their biological and toxic activities remain in most cases undetermined. The widespread distribution of these toxins in seafood has caused increased concern due to the threat to public health and has stressed the need for appropriate detection methods and toxicological studies to evaluate the potential risk for human health when they enter into the food chain. DSP toxins used in this study (> 95% purity) were obtained from clonal cultures of the dinoflagellate Prorocentrum belizeanum after extraction and chromatographic purification. Male mice were used in this study. Each specimen was randomly assigned to be administered one of seven different doses (1 to 13 micrograms/mice) of toxin via a single intra-peritoneal injection. Each concentration and the control were repeated ten times. 24 hours after the injection, animals were euthanized and brain, heart, liver and adipose tissue from each animal were biopsied and immediately stored a -80ºC. 1H NMR spectroscopy of the aqueous-soluble extracts of each tissue have been performed on a Bruker Avance III 600 spectrometer. The aim of this study is to analyse the acute effects of different DSP toxin concentrations in different tissues using animal models. Therefore, different doses (1 to 13 micrograms/mice) of toxin were administered to mice via a single intra-peritoneal injection. Subsequently, lethal dose (LD50) values for a non-regulated/no-controlled toxin (7-DOA) were determined and found to be very similar to that of the lead toxin okadaic acid. Untargeted metabolomics of mice brain, heart, liver, and adipose tissue aqueous-soluble extracts were analysed by >1</sup>H-NMR using standard 1D solvent suppression, NOESY and CPMG pulse sequences. Principal component analysis of the spectra shows separation upon injection of different toxin concentration. Metabolic profiling of mice tissues showed changes in metabolite concentrations, which refer to increasing toxin concentration that may be related with disruption of different metabolic pathways by inhibition of protein phosphatases. This is the first NMR metabolic profiling study about the toxicological effects of the okadaic acid class of toxins.

POSTER 214

MONITORING THE METABOLIC EFFECTS OF A NOVEL PROBIOTIC BY HIGH-RESOLUTION MASS SPECTROMETRY BASED METABOLOMIC FINGERPRINTING

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Probiotics are defined as 'live microbial organisms that exert health-beneficial activities for the host'. These activities are mainly targeted towards the gastrointestinal tract (GIT) and contribute to its microbial balance. Gut microbiota play a critical role in a wide range of diseases, such as inflammatory bowel disease, etc. Therefore, modulating the gut microbiome through the administration of probiotics offers a broad application range (prevention or even therapeutic). Unfortunately, knowledge on the mechanism behind the success of probiotics is limited. The aim of this study was to investigate the probiotic-induced metabolic effects and its mechanisms by implementing a metabolomic fingerprinting approach on samples derived from in vitro gut simulations. In this study, this new probiotic strain was supplemented to the dynamic SHIME model through different delivery strategies. This SHIME model (Simulator of the Human Intestinal Microbial Ecosystem) comprised the sequential simulation of stomach, small intestine and colon (i.e. proximal and distal). After a stabilization period of two weeks, the SHIME reactor underwent a control period of 10 days, followed by probiotic supplementations for 19 days. Prior to metabolic fingerprinting of the digestive fluids by means of UHPLC-HR-Orbitrap-MS, chemometric optimization of the extraction was performed to obtain the highest metabolite coverage. Finally, multivariate data analysis strategy was undertaken to investigate the probiotic-induced metabolic changes in the colon and evaluate the bioavailability of specific probiotic-produced metabolites through different delivery strategies. The development of a generic extraction procedure comprised a fractional factorial design. Of the five tested parameters, only the type of filter proved statistically relevant (P0.99) after omitting the highest level (i.e. undiluted). Therefore, an aqueous dilution (1:5) of the sample extract was necessary to comply to the optimal dynamic range of the MS. All precision measurements (i.e. instrumental, inter-assay and intra-assay) obtained CVs below the FDA recommended level of 15%. The differential analysis of the metabolic fingerprints from the proximal and distal colon digestive fluids revealed 3631 and 5257 monoisotopic ions, respectively. The intensities of the QC samples were used to construct a correction trace for the dataset prior to performing multivariate statistics. An unsupervised PCA plot already indicated a differentiation between the control and the treatment samples. Moreover it showed that the first sample of each period required some additional stabilization, as it was not located within its parent cluster. Finally, supervised validated (PANOVA<0.05) OPLS-DA models were constructed. The validity characteristics of these models R2(X), R2(Y), Q2(Y) were all well above the general accepted threshold of 0.5, acknowledging the predictive power of the models. A clear classification between the control and the probiotic supplemented samples was observed, even between the different delivery strategies, indicating a difference in bioavailability. Loading S-plots will be constructed to investigate the differences or similarities between the metabolic changes induced by the different treatments more in depth and elucidate discriminating metabolites. Metabolomic fingerprinting using UHPLC coupled to HRMS provides insights in probiotic-induced metabolic effects in dynamic gastrointestinal models.

POSTER 216

Assessment of metabolomic studies in monitoring the course of treatment of rheumatoid arthritis.

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Rheumatoid arthritis (RA) is a long-term disease in which joints in the body become inflamed, causing pain, swelling and stiffness. Common, non-specific symptoms and the lack of an easy method, for definitively proving or ruling out a diagnosis, makes early recognition very difficult. Blood testing is a widely used procedure in modern medicine. The ability to diagnose RA during such a common analysis makes serum a promising object of research.The aim of the study was to investigate the diagnostic potential of a metabolomics study, performed by NMR spectroscopy, chemometrics and statistical methods, in identifying RA and monitoring its progression in its early stages, where treatment is most effective. Serum samples were collected from female patients, with established rheumatoid arthritis, before and after 3 months of pharmacological treatment. They were also collected from healthy controls.The analysis of serum samples was performed using proton nuclear magnetic resonance (NMR) spectroscopy. All spectra were exported to Matlab for preprocessing, which resulted in data matrix. Further analysis included chemometrics partial least-squares-discriminant analysis (PLS-DA) and statistical testing (Student's t test and Mann-Whitney-Wilcoxon test). The comparisons between the group of patients with diagnosed RA and the healthy group provided good PLS-DA models. The metabolites that allowed these groups to be distinguished were i.a.: valine, isoleucine, lactic acid, alanine, creatinine, pyruvic acid and histidine. In addition, significant differences in metabolomic profiles were found between the group of patients before pharmacological treatment and after treatment. The strongest biomarkers were i.a: leucine, creatinine, trimethylglycine, phenylalanine, histidine and formate.It is difficult to compare these results with others because there are no other studies of similar groups of patients using serum as a sample and NMR spectroscopy as a method. However, there are a small number of studies which also indicate abnormally low level of histidine in RA patients.The results obtained confirm the potential of this method in the diagnosis and monitoring or rheumatoid arthritis. However, more studies are required. The promising results show the use of NMR spectroscopy with serum has potential as a novel method for RA diagnostics.

POSTER 217

Mechanism through which a remodeling of cellular metabolism delays aging in yeast

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Recent data have suggested that a characteristic age-related remodeling of cellular metabolism may play essential roles in regulating cellular aging, influencing age-related pathologies and defining organismal longevity in eukaryotic organisms across phyla. However, mechanisms underlying such remodeling and mechanisms by which longevity-extending dietary, pharmacological and genetic interventions regulate such remodeling remain unknown. Our research is focused on understanding molecular mechanisms through which an age-related remodeling of cellular metabolism defines longevity of the chronologically aging budding yeast Saccharomyces cerevisiae. Yeast were cultured in the nutrient-rich YPD media supplemented with 0.2% or 2% glucose. Cell cultures were harvested by centrifugation, washed with nanopure water, quenched with a MeOH:H2O (1:1) mixture and stored at -80°C. Cells were spiked with internal standards and then lysed to extract metabolites. The supernatant was concentrated using a SpeedVac system and then diluted with LC solvents. Data were acquired using an LTQ Orbitrap Velos mass spectrometer equipped with an HESI-II ion source following metabolite separation with the help of a ZIC-pHILIC column or a C18 reverse phase column. Metabolites were detected by top five data dependent FT-MS/MS. Samples were analyzed in both positive and negative ionization modes. MZmine and metaboanalyst.ca were used for data analysis. With the help of a mass spectrometry-based quantitative analysis of the entire cellular metabolome, we investigating how age-related changes in various pathways of central and intermediary metabolism within a chronologically aging yeast cell are influenced by several dietary, genetic and pharmacological interventions known to extend longevity in organisms across phyla. These interventions include: (1) a caloric restriction (CR) diet; (2) the tor1Δ mutation, which eliminates the Tor1 protein kinase known to orchestrate the nutrient- and energy-sensing TOR (target of rapamycin) pro-aging signaling pathway; (3) the ras2Δ mutation, which eliminates a GTPOSTER binding protein shown to activate the nutrient- and energy-sensing cAMP/PKA (cyclic AMP/protein kinase A) pro-aging signaling pathway; (4) the rim15Δ mutation, which eliminates the

nutrient-sensory protein kinase Rim15 integrating the TOR and cAMP/PKA signaling pathways; and (5) lithocholic acid (LCA), a natural anti-aging compound that we discovered. Our findings imply that all these anti-aging interventions exhibit similar effects on the intracellular concentrations of metabolites comprising a network which integrates the following pathways of intermediary metabolism: (1) the biosynthesis of methionine from aspartate; (2) the biosynthesis of threonine from aspartate; (3) the biosynthesis of both cysteine and glutathione from homocysteine, an intermediate in the biosynthetic pathway for methionine; and (4) the biosynthesis of spermidine and other polyamines from S-adenosylmethionine, an intermediate in the biosynthetic pathway for methionine. Based on these findings, we propose the following hypothesis: (1) an age-related redirection of metabolite flow within the network from the biosynthesis of methionine and spermidine to the biosynthesis of cysteine and glutathione is an anti-aging pattern characteristic of the "metabolic signature" of longevity extension in yeast; and (2) CR and LCA stimulate the development of such anti-aging metabolic pattern prior to the entry of a yeast cell into a quiescent state, whereas both the TOR and cAMP/PKA pro-aging signaling pathways inhibit it. A novel mechanism through which different longevity-extending interventions delay aging by modulating metabolite flow within a network of intermediary metabolism.

POSTER 219

Effects of a Simulated Work Week of Insufficient Sleep on the Human Plasma Metabolome

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Insufficient sleep has been shown to dysregulate glucose metabolism and reduce insulin sensitivity. Molecular mechanisms contributing to these negative metabolic consequences of insufficient sleep in humans are poorly understood. Therefore, we investigated changes in the plasma metabolome during a simulated work-week of insufficient sleep to identify potential mechanisms by which insufficient sleep contributes to metabolic dysregulation. Participants (N=16; 8 men/8 women, aged 22.4±4.8y[mean±SD]) completed a cross-over 14-15 day in-laboratory study consisting of 3 baseline days (9h sleep opportunities; BL) followed by a 5 day control condition (9h opportunities; 9-h) and a 5 day insufficient sleep condition (5h sleep opportunities; 5-h), counterbalanced. Blood was collected on the final day of BL, 9-h, and 5-h conditions. Protein precipitation and organic liquid-liquid extraction was performed on plasma to obtain aqueous and lipid fractions that were analyzed by untargeted LC/MS. In-house and public databases including METLIN, Lipid Maps, KEGG, and HMDB spectral data was used to annotate metabolites based on exact mass, isotope ratios and isotopic distribution with a mass error3000 counts. Oral glucose tolerance test derived insulin sensitivity decreased ($P \leq 0.05$) after 5-h versus BL and 9-h conditions. Metabolomics analyses were performed on fasted morning samples. A total of 3883 metabolites were annotated. Metabolites in ≥ 50% of samples for at least one condition (2737 metabolites) were analyzed. Using one-way Welch's ANOVA with Benjamin

POSTER 220

Brain Activity During Sleep: The Metabolic Connection

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The potential role of sleep as a restorative process raises the possibility that the dynamics of sleep can be quantified by profiling the circulating metabotype. Different stages of sleep have important physiological roles and can be followed by the brain response during each stage quantifiable by the electroencephalogram. Clinically, polysomnography(PSG) is used to score different stages of sleep. Metabolically however, little is known regarding metabolic changes with sleep state. Healthy

individuals were allowed to sleep for one night in a fully equipped sleep research facility. Polysomnography was used for scoring different sleep parameters overnight using epochs of 30 seconds. Serum samples were collected from each individual every two hours. The samples were profiled using 700 MHz NMR spectroscopy followed by quantitative targeted profiling and multivariate regression modelling. Principal component analysis and Orthogonal Partial Least Square (OPLS) regression modelling clearly suggests that the metabolic compositions of serum from the individuals are significantly correlated to the PSG scored sleep states. Further, the results also suggested that different sleep states and the degree of wakefulness are related to anabolic and catabolic activities. Such quantitative relation indicates that the sleep states and hence the brain activity during sleep could be assessed using metabotyping of the sleeper. Metabolic profiling could be utilized to unravel the underlying biology of brain activity during sleep as function of systemic metabolism.

POSTER 221

Exploring metabolic relevant pathways of frailty

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Frailty can be considered a progressive loss of reserve in multiple physiological functions which is primarily due to aging. In this study, we attempt to gain more insight into the biochemical alterations that characterize this clinical phenotype in elderly cancer patients using a targeted metabolomic approach The serum metabolomics profile of 89 elderly women with breast cancer clinically classified as Fit (n=49), Unfit (n=23), or Frail (n=17) according to comprehensive geriatric assessment . The metabolomics profile was determined by LC- MRM -MS/MS targeted to amino acids, acylcarnitines, sphingo- and glycerol-phospolipids The result of this investigation indicate that patients carrying the frail phenotype as compared to fit patients have significative perturbations of the amino acid pathways mainly involved serine, tryptophan, hydroxyproline, histidine, its derivate 3-methyl-hystidine, cystine and aminoisobutyric acid. Interesting, the frailty phenotype was found characterized by a significative decrease of a wide number of glycerol- and sphingo-phospholipid metabolites. These represent new dignostic biomarkers of frailty that may give new insight into the development of this complex phenotype .

POSTER 222

LC-MS based untargeted metabolomics approach to explore biomarkers of aging in human plasma samples

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Aging is a complex phenomenon involving gradual accumulation of diverse detrimental changes in biological systems and, as a result, significant changes in cellular metabolism are produced throughout the aging process. Normal aging process also often accompanies various diseases such as Alzheimer's, diabetes and chronic kidney disease. It has been proposed that aging process itself may be the underlying reason for these age-related diseases and slowing down or reversing it can potentially prevent or cure these conditions. Application of metabolomics technologies to study the aging is a relatively new field and may lead to biomarkers for the aging process. To investigate metabolic changes involved in aging process, plasma samples of healthy subjects of various ages, namely 40s, 50s, 60s and 70s, obtained from a local hospital in Singapore. After deproteinization, the samples were analyzed using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry in ESI positive and ESI negative mode. Pooled quality control samples were also analyzed during the run to check for system stability. After peak picking and statistical analysis, differential metabolites among groups were chosen and, after performing MS/MS studies, were searched against spectral databases for identification before carrying out pathway analysis for biological significance. Along with univariate statistics, multivariate statistical analysis of the data using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (O-PLS-DA) revealed separation among different age groups involved in the study. Metabolic features responsible for the separation between groups were chosen based on their variable importance for the projection (VIP) values and searched against accurate mass metabolite libraries of METLIN and Human Matabolome Database (HMDB) for identification. Pathway analysis on

the identified features revealed a number of metabolites that were involved in the lipid metabolism. The study provides new insights into the mechanism and biomarkers of aging process.

POSTER 223

Leveraging System-Wide Metabolomic and Transcriptomic Analysis to Improve Process Understanding of a Live Viral Vaccine.

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Process development of a live viral vaccine (LVV) depends on understanding the biochemical picture of the cell substrate during vaccine production. Moreover, current knowledge of our viral infection in human cells and the mechanism by which this virus alters and utilizes the host cell machinery for its production of viral progeny is poorly understood. Therefore, understanding the molecular basis of viral infection is of both fundamental and medical importance. To investigate the underlying mechanism of viral infection inside our cell substrate andimprove our LVV process understanding, we utilized system-wide transcriptomic and metabolomic techniques. This multiplatform analysis utilizes liquid chromatography tandem mass spectrometry (LC-MS/MS) / gas chromatography-mass spectrometry (GC-MS/MS) and nuclear magnetic resonance (NMR) spectroscopy metabolomics profiling to identify changes in the metabolic network of our cell substrate induced by virus infection. Culture samples were submitted for metabolome analysis across different culture models (static and suspension culture) and different scales during various times of virus infection.We also conducted genome-wide transcriptomic analysis to marry changes in gene expression to metabolite changes. In addition, we also conducted a carbohydrate and hormone screen to identify lead compounds that will have a positive effect on not only the metabolome but also the viral production process. The actual results will be discussed and the data will be presented at the meeting The results from this global analysis would help us to understand the systemic and metabolic alterations in our process

POSTER 224

Enteric fever metabolomics - comparing metabolite signatures of blood culture-positive and -negative samples

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Enteric fever is an infectious disease caused by the bacterial pathogens Salmonella Typhi and Salmonella Paratyphi A. The organisms are transmitted faecal-orally and induce a bloodstream infection after a brief period of gastrointestinal colonisation. The current diagnostic gold standard for enteric fever is blood culture, which is time-consuming and has inadequate sensitivity and specificity. Therefore, an increased understanding of host/pathogen interactions during disease is required for new diagnostic methods. Here, metabolomics analysis of blood plasma and urine from suspected enteric fever patients was performed to i) investigate metabolite signatures of blood culture-positive and -negative enteric fever, ii) validate results from a previous study of acute Typhi and Paratyphi infection and iii) compare metabolite signatures between plasma and urine samples. 30 matched plasma and urine samples from enteric fever patients in Bangladesh were analysed with a metabolomics approach including GCxGC/TOFMS and UHPLC/QTOFMS. The experimental design included patients with blood-culture confirmed Typhi infection (n=10), patients with negative blood culture but clinical suspicion Typhi infection through antigen detection and/or clinical symptoms (n=10) and a control group with no signs of infection (n=10). The plasma samples were analysed with both GCxGC/TOFMS and UHPLC/QTOFMS while the

urine samples were only analysed with UHPLC/QTOFMS. The relative metabolite concentrations of both identified and unidentified metabolites were subject to multivariate data analysis, to investigate and compare the metabolite signatures. Investigation of the plasma GCxGC/TOFMS data indicates that the Typhi culture positive samples can be distinguished from the control samples by means of OPLS-DA modelling. However, only weak models were obtained between Typhi culture negative samples and control as well as between culture positive and negative samples indicating a possible divergence within the culture negative group. This divergence was confirmed by predicting the culture negative samples into the model of culture positive versus control, revealing a few of the samples being predicted as culture positive samples while some being predicted as controls. Comparing the significant metabolites from this study associated with a culture positive confirmation of disease with significant metabolites from the previous study associated with acute Typhi disease highlights a large number of common metabolites significant in both studies. A faster and more reliable diagnosis of enteric fever would be of huge global health relevance.

POSTER 225

Integrated plasma and lung tissue metabolomics compled with LC/Orbitrap MS and chemometric analysis on potential biomarkers in RSV infected mice

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Respiratory syncytial virus (RSV) is among the most important pathogenic infections of childhood and is associated with significant morbidity and mortality. Rapid identification of this pathogen as a cause of infection would be important clinically, and could save many lives. Studying the host metabolic response to infection is a potential way of increasing our understanding of the RSV infectious process and of finding new approaches to diagnosis and treatment. Metabolomics offers a unique approach to characterize the host response by evaluating changes in accessible biological samples such as urine and plasma, as well as tissue. Here, an integrated plasma and lung tissue metabolomics strategy based on LC/Orbitrap-MS was used to capture the potential biomarkers in RSV infected mice. BALB/c mice were sensitized and inoculated intranasally once daily with RSV for 3 days to develop RSV infected pneumonia. Plasma and lung tissue samples were collected respectively 7 days after inoculation/treatment protocols, and prepared with an extraction based on methyl-tert-butyl ether (MTBE). We developed a LC/Orbitrap-MS, a high-resolution mass spectrometry, based on metabolomics method to measure the untargeted metabolites in the upper and lower phase of biological samples extracted by MTBE. Principal component analysis (PCA) and orthogonal partial least squares-discriminate analysis (OPLS-DA) were utilized to discover the differentiating metabolites in metabolomics data of plasma and lung tissue. In addition, histopathology of lung tissue was also examined. The mice model of RSV infected pneumonia was estabolished successfully and proved by the histopathological examinations. Subtle differences were identified by multivariate statistical analysis (i.e., PCA and OPLS-DA). In the positive modes, a total of 825 and 1004 signals, including upper and lower phase, were detected respectively in plasma and lung tissue samples. PCA and OPLS-DA models showed that RSV infection caused changes in metabolites of plasma or lung. Furthermore, we used S-plots to discover the potential biomarkers with variables (VIP>2) and statistical significance (t-test, $P < 0.05$). Consequently, 25 metabolites were selected as potential biomarkers in plasma and lung tissue respectively. Compared with normal mice, plasma levels of TG (18:1/18:2/18:2), TG (18:1/18:1/18:2), PC (18:1/22:6), and PC (18:0/20:5) were significantly up-regulated in RSV infected mice ($P<0.05$), while the other 21 lipid metabolites were significantly down-regulated ($P<0.05$). For lung tissue, a total of 23 lipid metabolites and 2 amino acids were all significantly down-regulated ($P<0.05$). Functional pathway analysis revealed that the alterations in these metabolites were associated with glycerophosphocholine metabolism, triacylglycerol metabolism, sphingolipid metabolism and amino acid metabolism. Metabolomic study of plasma and lung tissue offers a promising approach to investigating RSV to discover new pathogenic molecules, therapeutic targets and effective

pharmacological agents. Our overall findings revealed considerable pathway changes in metabolome in RSV phneumonia, including lipid and amino acid metabolism.

POSTER 226

The Microbiome and Metabolome of the Vaginal Mucosa Is Associated with Susceptibility to HIV-1 Infection

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The vaginal microbiome (VMB) plays a critical role in determining the vaginal status in women, healthy or dysbiotic, can affect susceptibility to HIV-1 infection via vaginal intercourse, and can affect the safety and efficacy of topical microbicides for HIV-1 prevention. However, the mechanisms that underpin the signaling between vaginal microbiota and the mucosa, their metabolomes, and the consequences to HIV-1 prevention remain poorly understood. Here, we use a novel, ex vivo human vaginal epithelial cell (VEC) multilayer tissue culture model, colonized with complex VMBs, to investigate the host-bacteria relationships in terms of their metabolomes and how these are associated with susceptibility to HIV-1 infection. Vaginal microbiomes, characterized by culture-independent methods, in cryopreserved specimens collected from women undergoing routine gynecological exams were used to colonize the air-interfaced apical surface of our novel cultured vaginal mucosae that include HIV-1-susceptible cells. The VMBs represented the six major community state types (CSTs) found in healthy women as well as several from women clinically diagnosed with bacterial vaginosis (BV). The microbial communities developing in the cultures were characterized by molecular methods. The metabolites present in the apical secretions and intracellularly in the VECs/ HIV-1-susceptible cells were analyzed by two complementary derivatization GC-MS methods and cryoprobe 1H NMR spectroscopy at 800 MHz. Associations between the metabolomes, VMB composition, and susceptibility to HIV-1 infection were analyzed using multivariate statistics. The VMBs in the ex vivo culture system faithfully recreated the complex communities that colonized the vaginal mucosa of the original donors. Bacterial CSTs were linked with susceptibility to HIV-1 infection. Lactobacillus-rich communities afforded protection from HIV-1 infection, while selected dysbiotic VMBs representative of BV increased susceptibility. The corresponding metabolomes, as measured by two complementary derivatization GC-MS methods (one broad spectrum technique and one specific to volatile carboxylic acids) and high field 1H NMR spectroscopy, were carefully interrogated for specific signaling molecules including volatile carboxylic acids, as well as profiling nontargeted metabolites. Multivariate statistical analysis in a principal component analysis space was applied to examine intrinsic clustering of the data. Select metabolites were identified as potential targets for further study. Metabolomics was applied to study host-bacterial community associations in our novel vaginal mucosa and associated with concomitant HIV-1 infection susceptibility.

POSTER 228

Insights into metabolic adaptation of Staphylococcus aureus and Burkholderia pseudomallei towards environmental stresses

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Microbial pathogens like Staphylococcus aureus and Burkholderia pseudomallei are emerging threats to

humans. Severe infections are caused by these bacteria including strains multi resistant to common antibiotics. In particular the urgent search for new antibiotics and the elucidation of the mode of action for new promising substance as well as the investigation of physiological adaptations of S. aureus and B. pseudomallei is a topic of the present study. Microorganisms need to preserve their function against a wide range of external perturbations; biotic or abiotic factors provoke cellular adaptations to maintain organisms in homeostasis. Microbial metabolomics plays a pivotal role for the understanding of the variability and dynamic of bacterial metabolism by measuring the direct biochemical response to the environment. Batch cultures of S. aureus and B. pseudomallei were analyzed in a time course experiment series. The extracellular metabolome was analyzed using NMR spectroscopy (600 MHz) with a special focus on amino acids and derivatives of the bacterial overflow metabolism. Using HPLC-MS and GC-MS methods intracellular metabolites were investigated to quantify unambiguously identified metabolites. Metabolite identification was verified by matching the retention times and fragmentation patterns of detected peaks to those of analytical standard compounds measured within the same batch. The investigation of highly charged compounds (e.g., (p)ppGpp) was done via HPLC-MS verified by matching the retention times and m/z values of detected peaks to those of analytical standards. The metabolome investigations were accompanied by in-vitro infection assays. Burkholderia pseudomallei is able to form various colony morphology variants on ashdown agar and is able to switch from one morphotypes to another in vitro as well as in vivo. The impact of this behavior on the metabolome showed distinct patterns in the investigated morphotypes. Nutrition uptake and metabolite secretion is comparable in the carbohydrate group but shows differences in the amino acid pool. The impact of these patterns on the virulence of different B. pseudomallei morphotypes could be reproduced in in-vitro infection studies. The global intra- and extracellular metabolic profiles of S. aureus were exploited to investigate the impact of antibiotic compounds with different cellular targets on the metabolome. Primary metabolism was largely covered, yet uncommon staphylococcal metabolites were detected in the cytosol of S. aureus, including sedoheptulose-1,7-bisphosphate and the UDPOSTER MurNAc-pentapeptide with an alanine-seryl residue. For each antibiotic compound, accumulation as well as depletion of metabolites was detected, often comprising whole biosynthetic pathways, such as central carbon and amino acid metabolism and peptidoglycan, purine, and pyrimidine synthesis. Ciprofloxacin altered the pool of (deoxy)nucleotides as well as peptidoglycan precursors, thus linking stalled DNA and cell wall synthesis. Fosfomycin inhibited the first enzymatic step of peptidoglycan synthesis, which was followed by decreased levels of peptidoglycan precursors but enhanced levels of substrates such as UDPOSTER GlcNAc. In contrast, vancomycin and ampicillin inhibited the last stage of peptidoglycan construction on the outer cell surface. As a result, the amounts of UDPOSTER MurNAc-peptides drastically increased, resulting in morphological alterations in the septal region and in an overall decrease in central metabolite levels. The responses of S. aureus to mupirocin and a new isoquinoline antibiotic could als be tracked. In the case of mupirocin treatment (inhibition of the bacterial isoleucyl-tRNA synthetase), S. aureus cells showed accumulation of branched-chain amino acids, adenosine nucleotides and the alarmone ppGpp. Staphylococcus aureus and Burkholderia pseudomallei show distinct metabolic adaptation patterns as found in metabolome analysis

POSTER 229

The mammalian antimicrobial metabolite itaconate drives inflammation by inhibiting succinate dehydrogenase (SDH)

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To remove and to protect itself against harmful stimuli, e.g. bacteria, cells activate their innate immune system resulting in drastic changes of cellular metabolism. Recently, the antimicrobial metabolite itaconate was identified in immune cells synthesized by cis-aconitate decarboxylase (CAD/IRG1) only under inflammatory conditions. Another recent study found succinate to be an important metabolite in the inflammatory response enhancing interleukin (IL)-1β production through stabilization of HIF. But how this metabolite accumulates during inflammation is poorly understood and is an area of intense interest. Here,

we demonstrate a new molecular mechanism for succinate accumulation: Under inflammatory conditions, macrophages produce itaconate to inhibit succinate dehydrogenase (SDH) resulting in increased succinate and IL-1ß levels. We used gaschromatography-mass spectrometry (GC-MS) metabolomics based techniques in combination with stable isotope-assisted metabolomics to demonstrate itaconate and succinate metabolism under inflammatory conditions. To elucidate the metabolic correlation between itaconate and succinate levels, we performed Irg1 gain- and loss-of-function experiments in various cell models, including resting and LPS-activated murine RAW264.7 macrophages as well as human A549 lung cancer cells. Along with the metabolite extraction, we isolated RNA and performed RT-PCR to directly compare changes in gene expression levels with metabolic changes. To analyze the effect of itaconate on mitochondrial respiration and SDH activity, we used the Seahorse XF Analyzer to measure the oxygen consumption rate (OCR) in permeabilized adherent RAW264.7 macrophages exposed to increasing itaconate concentrations and various substrates. Accumulation of succinate and itaconate in LPS-activated macrophages is well known, but a mechanistic link connecting accumulation of these metabolites has not been shown before. Performing Irg1 gain- and loss- of function experiments to affect itaconate concentrations in immune and non-immune cells, we show for the first time a direct correlation between itaconate and succinate levels. We exposed macrophages as well as non-immune cells to increasing extracellular itaconate concentrations resulting in increasing intracellular itaconate and succinate levels confirming the correlation between these metabolites. The observations so far strongly suggest that itaconate plays a major role for succinate accumulation under inflammatory condition and that succinate accumulation is independent on CAD/IRG1 and, thus, not specific to immune cells. To exclude a potential internally degradation of itaconate to succinate we applied stable isotope-assisted metabolomics on macrophages. Our results demonstrated that itaconate is not metabolized to succinate suggesting that succinate most probably accumulates in mitochondria due to decreased SDH activity. Although the inhibitory effect of itaconate on SDH has already been shown in-vitro in 1964 its role for succinate accumulation in mammals under inflammatory conditions has not yet been addressed and is unknown. To elucidate if itaconate inhibits SDH activity, we determined the influence of this metabolite on mitochondrial respiration in macrophages. We measured decreased OCR with increasing itaconate concentrations and confirmed that itaconate specifically inhibits SDH while having no effect on other mitochondrial respiratory chain complexes. Since it has been proposed that succinate stabilizes HIF1 enhancing IL-1ß production, we exposed macrophages to extracellular itaconate resulting in increased IL-1ß levels suggesting a regulatory role of itaconate for IL-1ß production. Our results clearly demonstrate the importance of itaconate as SDH inhibitor for intracellular succinate accumulation leading to increased IL-1ß expression. Demonstration of a direct mechanism for succinate accumulation under inflammatory conditions via the antimicrobial metabolite itaconate catalyzed by CAD/IRG1.

POSTER 230

Understanding the interaction of the pathogenic intracellular bacterium Coxiella with the mammalian host: A metabolomics perspective

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Coxiella burnetii is an intracellular bacterium responsible for the zoonotic disease Q fever. Infection in humans is acute or chronic, with a recent outbreak in the Netherlands costing over 300 million Euros to control and causing long-term health effects in some patients. Until recently Coxiella was considered an obligate intracellular organism, hampering attempts to characterise its pathogenic mechanisms. However the publication of axenic culture methods in 2009 facilitated the development of genetic manipulation methods for Coxiella. The ability to culture Coxiella outside host cells has now enabled us to optimise

methodology for applying metabolomics to Coxiella, both to dissect its central carbon metabolism, and to use metabolomics as a tool for functional characterisation of genes required for intracellular growth. C. burnetii Nine Mile phase II strain RSA493 was axenically cultured in liquid ACCM-2 at 37°C under microaerophilic conditions. During optimisation of gas chromatography-mass spectrometry (GC-MS) methodology, dry ice/ethanol bath and cold methanol infusion quenching were trialled. Cryomill extraction and freeze-thaw cycling were tested for cell lysis. Metabolites were extracted and partitioned into polar and non-polar fractions using chloroform/methanol/water before derivitisation of polar metabolites and GC-MS analysis. Metabolites were identified by comparison to mass spectral libraries. 13C-glucose labelling was carried out by addition of 3.33mM 13C-glucose to conditioned media and incubation for various time periods. The Coxiella cbu_0364 transposon mutant used here was constructed previously via electroporation of pKM225. Intracellular replication assays were carried out in mammalian HeLa cells. Initial optimisation experiments revealed that dry ice/ethanol bath quenching produced denser metabolite profiles than cold methanol infusion, suggesting that addition of the methanol directly to the Coxiella cultures resulted in premature cell lysis and loss of intracellular metabolites. Freeze-thaw lysis and cryomill lysis produced similar metabolite profiles. As cryomill lysis requires additional equipment, and has a greater likelihood of errors, freeze-thaw lysis was selected for use in future studies. Analysis of biological replicates (n=8) demonstrated the reproducibility of the chosen techniques. GC-MS was then used to compare 6 day cultures, considered to represent the replicating, metabolically active form of the bacteria (large cell variant; LCV), with 20 day cultures that are representative of the non-replicating form of the bacteria (small cell variant; SCV). Historically SCVs are considered to be metabolically inactive, and multivariate analysis demonstrated differing overall metabolite profiles from the two culture stages. Glycolytic intermediates were higher in the 6 day cultures, whereas tricarboxylic acid (TCA) cycle intermediates showed differing patterns. Initial 13C-glucose labelling experiments confirmed the labelling of intermediates in both glycolysis and TCA cycle, confirming these pathways are active in both culture stages. Incorporation of labelled carbon into glucose-6-phosphate was particularly important, as Coxiella lacks both hexokinase and some components of the alternative PEPOSTER phosphotransferase system. Despite this, our data clearly indicates that Coxiella metabolise glucose using glycolysis and the TCA cycle, and suggests that 20 day cultures are metabolically active. Optimisation of quenching and metabolite extraction methods for Coxiella now allows us to use metabolomics to investigate gene function. Previous screening of a Coxiella transposon mutant library for defects in growth inside mammalian HeLa cells identified cbu_0364, which encodes a putative general transport protein. GC-MS analysis comparing the cbu_0364 mutant with wild type identified a number of significantly different compounds, which will inform future transport assays. This is the first application of metabolomics technology to understanding Coxiella pathogenesis, opening up many new avenues for investigation.

POSTER 231

Investigating metabolic control in Plasmodium falciparum.

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Plasmodium falciparum, the deadliest malaria parasite adopts an assortment of forms during its life cycle and succeeds in very different host environments. A ‘just-in-time’ transcription, ensures that genes are only expressed when needed, allowing the parasite to develop and transit between hosts. Hence, rapid responses to the environment/diet and progeny adaptation ought to be promoted by metabolic changes. P. falciparum presents stage-specific changes in metabolic fluxes. During the intra-erythrocytic asexual stages glucose breakdown in glycolysis does not follow catabolism by the Krebs cycle. Instead, high rates of lactic fermentation are observed. We hypothesise that (a) glycolytic intermediates support rapid proliferation by their redirection into anabolic reactions and (b) flux to lactate could provide an effective strategy to control biomass production. By using Nuclear Magnetic Resonance (NMR) spectroscopy of 13C isotopes, we have been able to identify key glucose-derived catabolic products. The

parasitic nature of Plasmodium hinders intracellular studies over the whole life cycle, thus we have pursued analysis of extracellular material in order to infer metabolic changes in the fluxes. Using a new approach combining NMR metabolomics and imaging analysis we have related biomass production to the consumption and excretion of the most abundant metabolites over the entire 48h intra-erythrocytic life cycle of P. falciparum both qualitatively and quantitatively. The analysis pipeline has been coded in R and will be released for public use. A variety of nutrient conditions have been used to assess the interplay between metabolism and biomass. Culture media after incubation with parasites at various stages of development has been analysed. Glucose consumption increased slightly with the growth of the parasites. However, the amount of lactic acid excreted sharply increased from the initial life stages to the latest. These findings fit with our hypotheses suggesting that a bigger part of the glycolytic intermediaries are redirected to biomass in the earlier life stages of the parasite and less when the parasite has already grown. We have performed Principal Component Analysis (PCA) on the media metabolic read outs and showed differential clustering between infected and non-infected erythrocytes and also amongst the various stages of the parasite, emphasizing the prospects of metabolomics for diagnostics. To further test the hypotheses, we have developed culture medium that mimics physiological conditions, typically less rich than the usual culture media. We have discovered that parasites growing in this medium present statistically different phenotypes, with smaller areas, circularities and solidities. These parasites, which might seem to have a less healthy appearance, are slightly less susceptible to some of the antimalarial drugs. Whether this environmental adaptation is directed by metabolic changes must be answered. Parasites growing on the different culture media have been analysed over the entire intra-erythrocytic life cycle. Our experimental design consists of 16 time points in which we have taken metabolic read-outs, thin films for bright field microscopy analysis and samples for high content imaging in order to infer a relationship between the metabolites consumed/excreted and the biomass produced. We would like to present these exciting results. Innovative approach combining NMR metabolomics and image analysis of malaria to study metabolic control strategies analogous to cancer cells.

POSTER 232

Untargeted Metabolomics of CNS Disease During HIV-infection Using Humanized Mice

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A complete mechanism of HIV-associated neurocognitive disorders (HAND) remains an unmet gap in neuroAIDs research and therapeutic interventions, which are needed for medical management of HIV infection. The humanized mouse model provides a platform to study the pathways of neural demise during infection and treatment. HIV infection of humanized mice recapitulates progressive disease characteristics including immunology, behavior and neuroimaging. Moreover, this animal model permits the implementation of novel technologies to measure neural activation during HAND. The recent development of global metabolome profiling of brain tissue provides a unique opportunity to characterize novel biochemical pathways in health and disease. Brain metabolomics was implemented to capture dysregulated metabolite profiles of HIV infection. NOD/scid-IL-2Rgcnull (NSG) mice were reconstituted with human CD34+ hematopoietic stem cells, infected with HIV-1ADA and blood was analyzed for immunopathology and viral replication levels. In vivo magnetic resonance spectroscopic measurements of metabolites were acquired in the cortex and hippocampus at preinfection and after 12 weeks. Mice were then microwave irradiated in a Muromachi Microwave Fixation System to preserve brain tissues for metabolome profiling. Metabolomes of brain tissue subregions were extracted using a MeOH:H2O (4:1, v/v) solvent mixture and analyzed on Agilent 6550 iFunnel QTOF mass spectrometer interfaced with Agilent 1290 UPLC system using Phenomenex Luna Aminopropyl HILIC column and uploaded to XCMS Online web platform for data processing, including peak detection, retention time correction, profile

alignment, and isotope annotation. HIV infection of CD34+ humanized mice provides a model of progressive brain disease similar to human disease. This was modeled by behavioral and metabolic changes seen with memory deficits and decreasing NAA in the cortex at 12 weeks of infection. Focused beam microwave irradiation (FBMI) was validated for tissue preservation for both metabolite and protein measurements. We designed a heat sensitive phantom to test and tune FBMI heat distribution and intensity. FBMI heat fixation preserved metabolite levels in brain tissues and was necessary to stabilize specific metabolites. The LC analytical strategy of hydrophilic interaction enabled the MS detection of lipid and water soluble metabolite features across all brain regions, including the highly abundant, well-characterized brain metabolites (e.g. ATP, glutamate, taurine, NAA, NAAG), as well as lipids, phospholipids, central carbon metabolites, nutrients and metabolic by-products of cellular metabolism, including nucleotides, amino and non-amino organic acids. Using two-group analysis, HIV vs Control, for each brain region processed (hippocampus and dorsal cortex) we characterized the metabolite features whose variation pattern was statistically significant. Untargeted profiling in hydrophilic interaction mode, enabled the identification of differentially expressed metabolites with significant differences ($p \leq 0.01$). The cortical metabolome of HIV infected humanized mice had a significant reduction of ceremide species, creatinine and nucleobases. Additionally, cholesterol levels were increase during HIV infection. Global metabolomics were applied in an animal model of neuroAIDs thus providing a platform to develop neuroprotective therapy.

POSTER 233

Enteroviral Protein Induces Anomalous Changes in Host Cell Metabolism

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Enteroviral infection causes a major health problem in Asia-Pacific region. We have previously shown that infection with enterovirus 71 (EV71) leads to mitochondrial anomalies and energy metabolism. It is plausible that EV71 changes host cell metabolism to serve it need. To explore the possibility that virus changes host metabolism globally and exploits it to its own benefit we applied the UPLC-TOF-MS to examine the global metabolic changes in EV71 infected cells. There were significant changes in metabolome of the infected cells as compared with that of control cells. Intracellular levels of glutamine and glutamate specifically increased after infection, suggesting the glutamine-metabolizing pathways may be specifically affected by infection. Knockdown of the expression of CAD gene alone and of GDH and GLS genes in combination drastically reduces viral replication. Overexpression of CAD gene enhanced viral replication. Such findings advocate that CAD is critical to viral replication. Moreover, using reciprocal immunoprecipitation approach, we showed that enteroviral capsid was co-immunoprecipitated with CAD. This raises the possibility that enteroviral capsid protein interact with CAD to alter the glutamine-metabolizing pathway in a way to promote enteroviral replication. Our study gives an insight into interactions between virus and host metabolism.

POSTER 234

Combined multi-omics sample extraction and pathogen inactivation for systems biology studies of viral and bacterial infection

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Infectious diseases continue to be a major public health threat as globally recurrent (e.g., influenza and West Nile viruses), rapidly expanding (e.g., Ebola virus), and newly emerging (e.g., MERS-CoV virus) pathogens circulate widely among human populations. The development of predictive models of infectious disease initiation, progression, and outcomes requires study of dynamic host-pathogen molecular interactions using systems biology approaches. When working with highly infectious pathogens, great importance must be placed on safe, rapid, and effective inactivation of pathogens while at the same time generating high quality extracts for downstream mass spectrometry (MS)-based proteomics, metabolomics, and lipidomics analyses. Here, we present a modified chloroform/methanol procedure allowing for simultaneous pathogen inactivation and extraction of proteins, metabolites, and lipids for multi-omics analyses. Ten lipid-containing pathogens (4 viruses, 4 Gram-negative and 2 Gram-positive bacteria, and 1 spore-forming bacteria) were treated using a modified Folch extraction, which uses chloroform, methanol, and water (8:4:3, v/v/v) to induce a bi-phasic separation and subsequent protein, metabolite, and lipid (PML) extraction. Twenty replicates of each sample were treated with chloroform/methanol or buffer as the control for ≤5 min and evaluated for viable pathogen. To demonstrate simultaneous pathogen inactivation and extraction of PML of sufficient quality for subsequent multi-omics analyses, human epithelial lung cells were mock- and virus-infected with wild-type (A/Anhui/1/13), attenuated (A/Anhui/103F-106M) and enhanced (A/Anhui/691) H7N9 influenza virus strains (n = 5, each) at 6 time points. Samples were collected and analyzed using MS-based proteomics, metabolomics, and lipidomics. Chloroform/methanol treatment was shown to inactivate all pathogens, both viral and bacterial, within 5 min of exposure except for the gram positive bacterium Staphylococcus aureus, which showed a 5% survival rate, and Clostridium difficile spores, which are notoriously difficult to inactivate and showed a 2 log reduction in spore counts. A total of 41,340 peptides (corresponding to 5,296 proteins), 80 metabolites, and 440 lipid molecular species were identified in the mock- and influenza-infected human epithelial lung cells. Significant differences ($p < 0.05$) were found in proteins involved in many biological processes, including central carbon metabolism. Identified metabolites included amino and organic acids, carbohydrates, and polyamines. Identified lipids covered 3 lipid categories (glycerolipids, phospholipids, and sphingolipids) and 19 lipid subclasses, with PC, PE, PE-P, PI, and TG being the most abundant. Both protein and lipid results showed changes between the early (0 and 3 h) and late (18 and 24 h) time points, corresponding to time frames of viral entry, initial viral protein translation, and initiation of cell death. Unique changes also were noted with duration of infection and virulence type in all three omics measurements, offering insight into the role of various proteins, metabolites, and lipids in influenza infection. This protocol inactivates contagious and deadly pathogens while simultaneously generating suitable extracts for high quality multi-omics analyses.

POSTER 235

H NMR based metabolomics analysis of plants with antiviral activity from the African continent

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Viral diseases pose threat to many African countries with diseases such as HIV/AIDS, Herpes simplex and Ebola responsible for millions of deaths annually. Due to the extent and large numbers infected with

the diseases, current treatments are found to become ineffective as resistant strains are emerging continuously. Many plants on the African continent have been investigated for their possible anti-viral activity on various viruses such as measles, cytomegalo virus, HIV/AIDS and Herplex simplex. There is however very little information available on the chemical profile and constituents of the plants responsible for the anti-viral activity. There are however some indications from isolated and identified compounds, that there might be similar compounds or compound groups with anti-viral activity to be explored further. 600 MHz H-NMR analysis were used for metabolomics analysis of the 25 collected plant species. A direct extraction method was used and the data was analysed using MestReNova and SIMCA software. The parreto scaled data was analysed to determine the similarity index between plants with similar anti-viral activity. Specific focus was on regions for compounds such as dicaffeoylquinic acids and cardiac glycosides with proven anti-viral activity isolated from various plants for different anti-viral applications. Separate groupings were obtained for plants with anti-HIV, anti-CMV and plants without anti-viral activity. The compounds responsible for the groupings were further investigated to obtain information regarding anti-viral activity of poorly researched African plants. H-NMR based metabolomics is applied to assist or replace expensive bioassay guided fractionation techniques and regarding anti-viral compounds.

POSTER 236

Clofazimine Alters Host Energy Homeostasis that Involves Choline, Glycine and Methionine Metabolism

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Clofazimine (CFZ) is a rhiminophenazine antibiotic that is part of the World Health Organization's essential list of medications. It is an FDA-approved anti-mycobacterial agent for treating leprosy, and used off-label to treat other mycobacterial infections. Prolonged administration of this highly lipophilic drug results in a number of consequences including accumulation of CFZ in tissues that leads to the formation of intracellular drug crystals (IDC) that occurs over an 8-week period in mice. The underlying mechanisms of IDC formation are not fully understood but the crystals are surrounded by membranes likely of biological origin. To gain insight into potential metabolic mechanisms associated with CFZ-induced phenomena such as IDC formation, we conducted an untargeted metabolomics study in mice. Male mice (4 week old, C57BL6) were subjected to 18h metabolic studies, which included urine collection and measurement of weight and food and water intake, prior to and at 2, 4 and 8 weeks after treatment with either regular chow (sham) or regular chow supplemented with CFZ. At 8 weeks, whole blood (WB) samples were collected. The aqueous phase of MeOH:CHCl3 extracted WB and clarified urine samples were assayed by 1D-1H-NMR. Resulting spectra were processed and metabolites were identified and quantified using Chenomx software. Normalized data were analyzed by PLS-DA followed by ANOVA and t-test with false discovery rate correction (q) of the quantified urine and WB metabolite data, respectively, to compare sham and CFZ treatments. Mice tolerated CFZ with no apparent toxicity. Discrimination of CFZ and sham treatments was evident by PLS-DA of 51 urine metabolites at 2 weeks; choline had the highest VIP score. Analysis of temporal changes associated with CFZ treatment showed an abrupt increase in median [IQR] urine choline at 2 weeks (15.9 [14.0-19.4] vs.0.40 [0.36-0.44] μmole; q=0.006) followed by a precipitous decline that remained elevated compared with sham treated mice at 8 weeks (3.0 [2.5-4.3] vs. 0.36 [0.30-0.45] μmole; q=0.006). This trend coincided with urine glycine and methionine such that by 8 weeks, the amounts of these metabolites were lower than those of sham-treated mice (glycine: 1.0 [0.85-1.3] vs. 1.6 [1.6-2.4] μmole; q=0.006; methionine: 0.18 [0.11-0.26] vs. 0.82 [0.64-1.0] μmole; q=0.006). Of the 53 WB metabolites, median (IQR) concentrations of choline and glycine were higher in CFZ-treated mice at 8 weeks (choline: 135.7 [102.9-160.6] vs. 40.5 [26.9-50.2] μM, q=0.006; glycine: 369.7 [274.2- 484.1] vs. 169.5 [93-249], q=0.004); methionine was not different.

Glucose levels were not different between CFZ and sham treated mice (5717 [4209-6990] vs. 6442 [5559-8648] μM, q=0.56) but, on average (+S.D.), over the 8-week study, CFZ-treated mice ate 10% more food than sham-treated mice (2.6+0.5 vs. 2.3+0.6 g, p=0.05) and weighed less at 8 weeks (24.0+1.8 vs. 26.2+1.8 g, p=0.0002). Although 8-week urine volume was less in CFZ-treated mice, the rate of urine creatinine excretion, as a measure of renal function, was not different between the two groups over the course of the study. Urine metabolites associated with gut microbial disruption such as TMAO and TMA initially increased in CFZ-treated mice but were not different from sham-treated mice at 8 weeks. Additionally, of potential urine microbial derived metabolites, only indoxyl sulfate declined over time in CFZ mice. Apparent CFZ-induced conservation of choline may contribute to altered energy homeostasis that is associated with CFZ-induced changes including IDC formation.

POSTER 237

Determining mechanisms of cholera toxin using a rapid, comprehensive metabolomics profiling strategy

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Biological and chemical agents exert their effects by targeting molecular pathways that are critical for cellular function. Newly developed threat agents pose a particularly significant risk; without knowledge of mechanism, effective countermeasures cannot be developed. Quantitative measurement of small molecules is essential to the understanding of molecular mechanisms responsive to toxic agents. In the current study, we have developed strategies to rapidly and accurately quantitate over 95% known human cellular metabolites. Initial studies have utilized cholera toxin (CTX), which is produced by the bacterium Vibrio cholerae. CTX results in overproduction of cAMP, activating protein kinase A and causing a downstream efflux of water and ions out of enterocytes. Results confirm known mechanisms of CTX and suggest new pathways of interest. SH-SY5Y neuronal cells were treated with cholera toxin following (1) a defined time course and (2) different dose concentrations ranging from 0.3 to 10 ug/mL. Metabolites were extracted from SH-SY5Y neuronal cells using an MTBE method that yields aqueous and lipid fractions. Chromatographic separation was achieved by HILIC for aqueous metabolites, and C18 (RP) for lipids. Samples were analyzed using positive and negative ESI-MS using TOF and Q-TOF platforms. Data analysis was performed using in-house developed workflows based on Profinder and Mass Profiler Professional software packages (Agilent Technologies). Differentially regulated metabolites were tentatively identified using iterative database searching. MS/MS was performed on differentially regulated compounds and compared to standards and/or MS/MS libraries for more rigorous identification. Early work using only a dose response strategy revealed 700+ compounds in the lipid fraction found to have ≥ 2-fold change (after statistical analysis) between dose points. Using stringent parameters, 54% of those compounds have tentative database identification and 31% can be assigned a molecular formula. Two GM1 gangliosides are found to decrease with increasing doses of cholera toxin, indicating endocytosis. Additionally, lactic acid increases drastically at a 10 ug/mL dose, paralleling the acidemia typical of cholera toxin infection. Current work shows 2,133 compounds in the lipid fraction and 1,145 in the aqueous fraction that are unique to the samples. After statistical analysis using ANOVA and a 2-fold change filter, 421 compounds in the lipid fraction and 127 compounds in the aqueous fraction were found to be significant in the time course experiments. In the dose response experiments, 144 compounds in the lipid fraction and 46 compounds in the aqueous fraction were found to be significant. In the lipid fraction of the time course experiments, 1,173 MS/MS spectra were acquired using successive exclusion lists. Targeted lists were also used to collect 434 additional spectra. In the lipid fraction of the dose response experiments, 1,495 spectra were acquired using exclusion lists. Also, 241 spectra were acquired using a targeted list from differential analysis. Tandem MS experiments are underway for the

aqueous fraction. Due to the large number of MS/MS spectra obtained, a streamlined workflow has been developed for analysis. MS/MS spectra are batch processed using the NIST pipeline. Raw data is filtered based on score and probability eliminate the compounds unlikely to be identified using MS/MS search tools, allowing one to focus on manually curating the best spectra. This is further detailed in other work from our laboratory (S. Walmsley). Our newly developed, rapid method for obtaining significant coverage of cellular metabolites was used to confirm mechanisms of CTX.

POSTER 238

An untargeted metabolomics approach highlights short and long term effects of bariatric surgery in humans

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Bariatric surgery is currently the most effective treatment of obesity, leading to significant improvements in patients' metabolism. One major aspect of bariatric surgery in obese subjects is the reduction of cardiovascular risk, but the underlying mechanisms are not fully understood yet. Metabolic changes which are assessed shortly after the surgery have already been described in humans but a long-term assessment of changes in the metabolic profile of patients is still lacking. The aim of this study was to identify and quantify relevant metabolic changes which occur shortly after the surgery and after one year in a long term follow-up. 132 serum samples from 44 patients were analysed before surgery, after hospital discharge and one year after surgery. Samples were measured by LC-MS with an Ultimate 3000 UHPLC system coupled to a high resolution mass spectrometer Q-Exactive. A data driven approach was applied to identify important features that describe short and long term effects of bariatric surgery. The feature selection included Random Forests and univariate statistics. Trend-patterns over time of identified metabolites were described using median intensities to observe relevant metabolic changes. From the initial metabolic fingerprint (1000 metabolites) we assessed 150 features which characterize short- and long- term metabolic changes. 40 of these characteristic features were identified including metabolites such as trimethylaminoxide and phenylalanine which have been linked to cardiovascular risk. The identified metabolites showed different trend-patterns over time, highlighting the importance of short and long-term metabolomics assessment after bariatric surgery. For the development of potential prognostic markers, metabolites with a negative or a positive median trend are better suited for further investigations rather than metabolites with an alternating zigzag course. This study links short-term and long-term metabolic changes after bariatric surgery by using an untargeted metabolomics approach.

POSTER 239

Metabolic diversification in human retinal pigment epithelium cells induced by hyperglycemic and hypoxic conditions: a model of diabetic retinopathy

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Diabetic retinopathy (DR) is one of the leading causes of visual loss. The study of the mechanisms leading to neurodegeneration is necessary to identify new therapeutic targets in the early stages of DR. Although the functions of the retinal pigment epithelium (RPE) are essential for the survival of retinal neurons, to date the effects of diabetes on the RPE have received little attention. Here we investigate the metabolic changes occurring in RPE cells exposed to hyperglycemic and hypoxic conditions, the two major components of the diabetic milieu. For this, we implemented a non-targeted metabolomics and fluxomics approach based on LC-qTOF MS, GC-qTOF MS and NMR in a human RPE cell line (ARPE-19) cultured under conditions of hyperglycemia and/or hypoxia. ARPE cells were maintained in culture for 21 days with 5.5 mM or 25 mM of D-Glucose, and during the last 24 hours cells were subjected to serum deprivation. Serum deprived media were prepared with 5.5 mM or 25 mM of either D-Glucose or D-[U-13C]-Glucose, and cultured in normoxic or hypoxic (1% O2) conditions. Each condition was run in triplicate. LC-MS data was processed using XCMS, and geoRge. The latter is an in-house script to handle LC-MS stable isotope-resolved metabolomics data. GC-MS data was processed using eRah, an in-house R package that allows automatic spectra deconvolution, alignment, and library matching of metabolites. Our results reveal that hyperglycemia rather than hypoxia impacts on the metabolism of RPE. Hyperglycemia induces an altered oxidative state in RPE cells as revealed by the GSSG/GSH ratio, which is linked to changes in some intermediates of the cysteine metabolism. Increased levels of glycogen, sorbitol and nicotinate ribonucleoside were also observed under hyperglycemic conditions. In line with these results, our fluxomics approach performed on the same cells showed a characteristic rearrangement of the stable isotope tracer, namely D-[U-13C]-glucose, in hyperglycaemic conditions through the polyol, pentose phosphate and glycogen storage pathways. In contrast, we found diminished flux through the TCA cycle, glutamate and glutathione metabolism either in hypoxia or normoxia. Interestingly, in some cases the pool sizes of metabolites were not in accordance with the stable isotope labelling results. For example, we observed dysregulated flux through the purine and pyrimidine metabolism while we did not found changes in abundance for most of the metabolites involved in such pathways.Finally, preliminary data confirms some of these findings in human vitreous humour from patients with non-proliferative and proliferative diabetic retinopathy. Metabolomics and fluxomics reveal different layers of complementary biological information in the study of retinal pigment epithelium cells.

POSTER 240

Discovery of potential biomarkers on metabolic syndrome in patients with acquired diabetes

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Metabolic syndrome is known as a combination of metabolic disturbances. The abnormality is mostly associated with diabetes, cardiovascular diseases and cancers. Recent interest has looked into enlightening the uncertainties as to how diabetes affects certain metabolic pathways in humans. With the emergence of high resolution metabolomics studies, a more in-depth understanding can be performed on certain diseases. It can also offer to suggest possible biomarkers which could be used in early detection of these diseases. In this regard, the researchers proposed an application of high resolution metabolomics in detecting low molecular-weight metabolites which could be used as potential biomarkers for the diagnosis aimed to detect the development of diabetes. The candidate patients were selected from a cohort study (KCPS-II) of around 150,000 Korean male subjects ages 40-49 years. Subjects were selected based on the following: 1st year, 5 patients with diabetes, and 15 without diabetes. Of the 15 without diabetes, 5 patients who developed diabetes each year were selected for 2nd and 3rd years of the study. Different m/z values representing metabolites from the samples were analyzed using quadrupole time-of-flight LC-MS. These m/z values were statistically analyzed to identify significantly expressed metabolites after false discovery rate adjustment at a significance threshold of q=0.05.Metlin database was used to name these metabolites and a pathway analysis was performed through the Kyoto Encyclopedia of Genes and Genomes (KEGG) database thereafter. Comparison was performed in four ways: 1) all samples from the 5 patients with diabetes and all samples from the 5 patients without

diabetes throughout the whole 3 years (G1); 2) samples from the 5 patients with diabetes and samples from the 15 patients without diabetes at year 1 (TDM1); 3) samples from patients who acquired diabetes and samples from the 10 patients without diabetes at year 2 (TDM2); and 3) samples from 5 patients who acquired diabetes and samples from 5 patients without diabetes at year 3 (TDM3). Significant metabolite features from Manhattan plots with FDR q=0.05 were seen to be 836, 173, and 40 for TDM1, 2, and 3, respectively. In a two way hierarchical clustering analysis, separation of the metabolic profile of diabetes (DM) and non-diabetes (non-DM) were clearly observed and discriminated from each other. Among the significant features, N-Glycosyl-L-asparagine (m/z 295.1150 [M+H]+), Urocortisone (m/z 365.2289 [M+H]+), Malonamoyl-CoA (m/z 835.1324 [M+H-H2O]+), 5,6,7,8–Tetrahydromethanopterin (m/z 741.2543 [M+H-H2O]+), Bilirubin diglucuronide (m/z 919.3333 [M+H-H2O]+), and Triglycerides (m/z 795.6809 [M+H-H2O]+) suggest correlation with diabetes. Further analyses of these metabolites are being done to confirm their relevance to diabetes and diabetes acquisition. With the identification of potential biomarkers which can be linked to diabetes, further studies could aid in the early diagnosis of diabetes or in understanding further how diabetes is acquired. High Resolution Metabolomics may pave way to the further understanding on acquiring diabetes.

POSTER 241

Metabolomic Analysis of Type 1 Diabetic Primary T Cells Using Isotopic Ratio Outlier Analysis (IROA) by LC-MS

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Type 1 Diabetes (T1D) is an incurable, auto-immune disease that results from the destruction of insulin-producing pancreatic beta cells by pathogenic T lymphocytes. These defective T cells can differentiate into CD4+ T cells that correlate with T1D progression. Of the few experimental designs targeted to identifying the metabolic profile of solely T1D, many incorporate animal models that fail to account for pathophysiological differences in humans. There is a need to better understand the metabolic and lipidomic signature of this disease using human samples. This work employs isotopic labeling LC-HRMS methodologies to identify the metabolic and lipidomic trends of immune dysregulation using primary T cells obtained from T1D patients compared to 1st-degree relatives and healthy controls. Jurkat cells were grown and labeled on 95% 13C-IROA media. Once confluent, Jurkat cells were pelleted via centrifugation, washed, and reconstituted in an ammoniated cell washing buffer. Equal aliquots of the 95% 13C-labeled Jurkat cells were spiked into approximately 1E6 T cells obtained from T1D patients, 1st-degree relatives, and healthy controls. Proteins were precipitated in each group using 80% methanol/water, the supernatant dried under nitrogen, and the metabolites reconstituted in 0.1% formic acid in water. Additionally, lipids were extracted from the metabolite supernatant using the Folch extraction method, dried under nitrogen, and reconstituted with isopropanol. The reconstituted metabolite and lipid extracts were analyzed by LC-HRMS in positive and negative ion mode at 70,000 resolution (m/z = 200). All LC-HRMS data were collected using a Thermo Scientific Q Exactive and initially processed by the Xcalibur Workstation software (version 2.2.44). Data processing was performed using the XCMS R script, MetaboAnalyst 3.0 online, and the ClusterFinder software. By incorporating the phenotypical IROA methodology, artifacts and noise resulting from the media and the isolation of the T cells were removed because only biologically relevant compounds incorporated the 13C label from the 95% 13C-IROA medium into their metabolome, resulting in a unique isotopic signature. Therefore, the 95% 13C Jurkat cells represented an internal standard that contained labeled metabolites and lipids for every compound present in T cells, correcting for sample preparation and instrument variability. A cellular metabolomics/lipidomics sample preparation workflow was optimized for LC-HRMS biomarker analysis of the primary T cells using the IROA methodology. The workflow included an evaluation of various cell rinsing buffers compatible with electrospray ionization and a comparison of lipid extraction methods for global lipidomic analysis. Incorporation of this sample preparation process allowed for the analysis of both the metabolite and lipid content from a single pellet. Principal component analysis (PCA), random forest

(RF), hierarchical clustering (HC), and other multivariate analyses were performed on the primary T cell patient data to distinguish m/z peaks with the greatest and most significant variation between the T1D and healthy control/1st-degree relative groups. PCA score plots show separation between T cells from T1D and healthy control patients/1st-degree relatives. RF and HC similarly distinguishes T1D patients from both control groups. Metabolites/lipids showing significant differences in both the patient T cells and the 13C labeled Jurkat cells will be searched against the Human Metabolome Database, LipidMAPS, and METLIN database for biological relevance. First study to compare potential metabolite and lipid biomarkers from T1D patient T cells using Isotopic Ratio Outlier Analysis (IROA).

POSTER 242

Characterization and Collision Cross Section Determination of Obesity Related Lipids Within Mouse Models Using Travelling Wave IMS-QTof Mass Spectrometry

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Obesity is a risk-factors associated with metabolic syndrome, causing excess body fat to be accumulated to the extent that it adversely affects health and life expectancy. It has been demonstrated that glucosylceramides play a crucial part in metabolic syndrome. The manipulation of the function of glucosylceramides with small molecule drug compounds has shown that symptoms can be negated. A previous multi-omic study showed differentiation between subjects treated with glucosylceramide synthase inhibitors. This work is an extension, providing additional characterization of the liver cell lipid complement using ion-mobility and associated collision cross section (CCS) databases, obtained with a novel geometry travelling wave IMS-QTof MS platform, for obese mouse models which have undergone treatment to prevent or revert obesity. Lipid extracts were generated from liver tissue originating from 3 obese control and 3 inhibitor treated mice. The extracts were separated over a 20 min reversed-phase LC gradient. Data were acquired using a data independent acquisition approach utilizing ion mobility, whereby the collision energy was switched between a low and elevated energy state during alternate scans. Simultaneously, experimentally derived CCS values using travelling-wave ion mobility (TWIM) were determined using previously published CCS values for singly charged polyalanine oligomers as the TWIM calibrant species. The acquired data were processed and searched using Progenesis QI and dedicated lipid compound databases, providing normalized label-free quantitation results with additional afford specificity of CCS measurement. Lipid extracts were prepared using 500 µL IPA/water (50:50), of which 2 µL were injected on-column and analyzed in triplicate. Samples were acquired in a random order with a QC comprised of all samples in equal amounts and injected every 5 injections. Data analysis was conducted with Progenesis QI for processing, compound database searching and CCS calculation. Interrogation of the LC-IM-DIA-MS data revealed over 5000 potential features for further investigation as a result of positive and negative ion acquisitions combined. Data were further interrogated using multvariate statistical analyses, which showed clear distinction between control and glucosylceramide inhibitor treated groups. OPLS discriminant analysis revealed 795 potential features that were of significant correlation and covariance. These 795 features were database searched resulting in 163 potential candidates. Identifications were scored according to mass accuracy, isotoptic fit, CCS and MS/MS fragmentation. A variety of synthetic lipid standards representing the most significant classes identified were measured to determine their CCS for reference and used to populate the in-house database providing additional stringency to the search. Additional filtering to curate the data was based on mass errors less than 2 ppm, fold change greater than 2, CCS tolerance within 5% and ANOVA Poster value less than 6E-06. This resulted in 15 significant identifications for a range of lipid classes including phosphatidylcholines, sphingomyelins, triglycerides and lysophosphatidylcholines. Ion mobility-derived CCS measurements allowed for improved specificity with the inclusion of drift time and therefore provided additional confidence to the identifications returned. Pathway analysis revealed lipid metabolism as a significantly perturbed pathway with mapping highlighted to diabetes and inflammatory responses.

TWIMS enabled IMS-QTof lipid characterization and CCS determination to enhance specificity for glucosylceramide synthase inhibitor treated subjects.

POSTER 243

From metabolomics to simple biochemical assay: 1,5 –Anhydroglucitol to facilitate non-invasive screening for diabetes

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Type 2 diabetes (T2D) is often asymptotic in early stages and may remain undiagnosed for many years, what in consequence significantly increase risk factors for developing comorbidities. Implementation of screening strategies can support early detection of T2D and improve patient outcomes. Available diagnostic methods, for instance fasting blood glucose level, glycated haemoglobin (HbA1c) or 1,5-Anhydroglucitol (1,5-AG), require blood samples collection, which limits screening options. Therefore, development of noninvasive strategies for patients screening should be considered. In our previous study we pinpoint 1,5-AG in saliva as potential noninvasive biomarker for T2D using metabolomics (Mook-Kanamori, D. O. et al.,JCEM, 2014) . Currently we are optimizing easy biochemical assay to be applicable for quantification of 1,5-AG in saliva to facilitate diabetes screening. Plasma and saliva samples have been collected from healthy individuals and patients suffering fromT2D, as we previously described (Mook-Kanamori, D. O. etal.,JCEM, 2014). Non-targeted metabolomics approach, based on gas chromatography and ultra-high performance liquid chromatography coupled with tandem mass spectrometry, was applied on 188 T2D patients and 181 healthy controls at Metabolon. The concentration of 1,5-AG in plasma and saliva samples was measured with commercially available GlycoMark assay, developed for plasma and serum samples and based on the absorbance of sequences of the enzymatic reactions. The 1,5-AG concentration was determined using Randox RXDaytona+ clinical chemistry analyzer, by comparing the point of calibration based on the kinetic change in absorbance of the reagent blank and standard. Osmolality was determined with Fiske210-microsample-osmometer. Metabolic profiling of saliva samples resulted in identification of 581 metabolites, among which 1,5-AG exhibited significant association with T2D and correlation with 1,5-AG in plasma samples ((Mook-Kanamori, D. O. et al.,JCEM, 2014). Thus, we are considering 1,5-AG in saliva as potential non-invasive biomarker for T2D. Nevertheless, implementation of metabolomics in analytical laboratories is still less feasible than application of biochemical assay, compatible with clinical analyzer, which is operated on a daily bases in analytical laboratories. Therefore, we selected GlycoMark kit, already applied for measurements of 1,5-AG in plasma and serum samples, in order to adopt it for measurements in saliva. Because 1,5-AG level in saliva is lower than in plasma, we investigated level of detection by introducing dilution series on 1,5-AG standard solution at maximal concentration of 50 µg/mL. Our results are in good accordance with manufacturer information showing limit of detection at 0.2µg/mL. We further investigated the coefficient of variation (CV) after measurement of 60 mixed saliva samples from diabetes patients and healthy controls to verify variation in measurements of sample in the same run as well as day to day variation. Additionally we estimated effect of freeze/thaw cycles on stability of 1,5-AG in saliva. Finally, we have measured concentration of 1,5-AG in saliva and plasma aliquots from subjects in which metabolomics profiling has been applied. We were able to distinguish diabetics from healthy controls based on 1,5-AG concentration in both plasma and saliva measured with GlycoMark kit. We have investigate the correlation between plasma and saliva samples after 1,5-AG measurements with biochemical assay, as well as the correlation of 1,5-AG estimated with metabolomics and GlycoMark kit. Our future goal is to estimate the threshold of 1,5-AG in saliva of diabetes and healthy individuals as well as to test whether children and adolescent present similar patterns. We present translation of metabolomics results for clinical application by adaptation of biochemical assay for 1,5 AG measurements in saliva.

POSTER 244

UHPLC-MS/MS characterization of reactive oxygen species and central metabolites in the regulation of cardiovascular function

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The occurrence and production of reactive oxygen species (ROS) are a major disease progression factor in the development of the metabolic syndrome. ROS levels found to be upregulated in hypertension and diabetes models. Under pathological conditions the endothelia NO-synthase (eNOs) which is normally producing NO from arginine to regulate vascular tone, are uncoupled and producing ROS as well. The activity of eNOS and the other enzymes involved in the aginine cycle, can be monitored by the levels of arginine, citrulline, and ornithine. Dihydroethidium (DHE) is known to form specific products in the presence of different reactive oxygen species. Depending on the predominant ROS species, DHE products allow the determination of the active ROS producing pathway. Cultured endothelia cells and 1mm aortic rings from diabetes and normal groups were treated with an inhibitor of nitric oxide biosynthesis (L-nitro-arginine methyl ester), and an activator of ROS production (acetylcholine) or a combination of both to target different ROS producing pathway. Cells or aorta were incubated with DHE, harvested in liquid nitrogen and homogenized. Metabolites and DHE were extracted in 50/50 (v/v) methanol/water. Specific DHE products were separated on a C18 column and detected by their specific MS/MS adducts on a QTrap5500 (ABSciex). Additionally, they were qualified by their exact masses on a QExactive (Thermo). Metabolite concentrations were quantified from the same sample by UHPLC-MS/MS on a QTrap5500. The presented DHE assay is sensitive enough to detect the specific DHE products in cultured cells as well as in 1 mm aortic rings. We can confirm the production of superoxide specific product like 2OH-ethidium, and different specific ethidium dimers in response to treatments known to induce superoxide production. Additionally, we identify a new DHE product specific to peroxynitrite. We found specific regulations of citrulline, arginine, and ornithine during eNOS inhibition in the diabetic model together with specific DHE products. Indicating an alteration of the arginine converting enzymes in ROS stressed endothelia cells. Our assay allows the detection of specific endothelia derived ROS species and tracing of metabolomics perturbation in the endothelium.

POSTER 247

Metabolomics approaches to find biomarkers of childhood obesity in Korea

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Childhood obesity is related to a wide range of serious metabolic complications, including insulin resistance, dyslipidemia, hypertension, and non-alcoholic fatty liver disease. The aim of study was to elucidate the changes in metabolites related to the childhood obesity. A total of 267 children aged 6 to 14 years [obese (n=72, BMI 24.2±2.9 kg m-20, overweight (n=38, BMI 21.6±2.4 kg m-2-, and normal controls (n=157, BMI 17.5±2.2 kg m-2), 147 girls and 120 boys] were recruited. An ultraperformance liquid chromatography and quadrupole time-of-light mass spectrometry (UPLC-QTOF/MS) was applied to urine sample obtained from subjects. Multivariate analysis was performed to identify metabolic markers and potential metabolic pathways associated with the obesity group separation. In positive electrospray ionization (ESI) mode, the most differential metabolites[a total of 399 (VIP scores > 4) out of 48582 variable ions] included 5-oxoproline and creatinine originating from proline metabolism, and cytosine from pyrimidine metabolism. In negative ESI mode, the most differential metabolites [a total of 364 (VIP scores > 4) out of 49224 variable ions] were cis-aconitate and 2-oxocaroxylic acid originating from citrate cycle, dopaguinone and dimethylbenzimidazole originating from tyrosine and riboflavin metabolism, respectively. From the library search, we found that pyrimidine metabolism was uPoster regulated, and

glutathione metabolism was down-regulated in obesity group. In conclusion, this study demonstrated that UPLC-QTOF/MS was a useful tool to validate a possible biomarkers and disturbed metabolic pathways in childhood obesity. biomarkers of childhood obesity in Korea

POSTER 248

Targeted Metabolomic Profiling of Bile Acids in Metabolically Active Tissues Reveals Reversible and Irreversible Effects of Obesity and Weight Loss

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Bile acids influence important bodily functions, including cholesterol catabolism, nutrient absorption, lipid/glucose homeostasis, liver and gut health. They consist of three classes (primary, secondary, and tertiary), each differing in the mechanisms and preferential locations of their synthesis/conversion and having unique metabolic and functional effects. In the context of obesity, disruption of bile acid signaling can influence insulin resistance and dyslipidemia. The effect of substantial weight loss after weight gain on bile acid metabolism is largely unexplored. In association with a larger study focused on lipidomics and lipid mediator analyses, we opportunistically utilized targeted metabolomic profiling to investigate alterations in bile acid concentrations of lean, obese, and lean mice that had previously been obese in plasma and several metabolically active tissues. Three groups of male C57BL6 mice (n=6/group) consuming either a: 1) high fat (HF) diet for 18wk to induce obesity; 2) low fat (LF) diet for 18wk to remain lean; 3) HF diet for 9wk to induce obesity prior to being switched (SW) to the LF diet for 9wk to induce weight loss. Liver, adipose, muscle, and plasma samples were analyzed for 31 bile acids. To isolate bile acids, tissues were cryopulverized in organic solvent with deuterium labeled surrogates, centrifuged, and supernatants were eluted through a 96-well phospholipid removal plate. Analytical targets were quantified by +ESI-UPLC-MS/MS on an ABSciex 4000 QTrap run in multi-reaction monitoring mode and quantified against authentic standards using internal standard methodology. Targeted analysis of the bile acid metabolome detected each class of these compounds in all measured tissues. Bile acid concentrations in the liver were 2-19 fold lower ($p<0.05$) in the HF group than the LF and SW groups for a variety of unconjugated and conjugated primary (CA, TCA, TCDCA, T- α-MCA), secondary (DCA, ω-MCA, T- ω-MCA), and tertiary (TUDCA) bile acids. These results suggest obesity-induced changes in the concentrations of these bile acids are reversed upon weight loss. However, concentrations in the SW group increased post-weight loss but were significantly different than both the HF and LF group in multiple secondary (TDCA, TLCA, GDCA) and tertiary (GUDCA) bile acid conjugates that are products of conversion by gut microflora. Thus, changes in the bile acid profile induced by weight gain may not fully return to lean-associated levels 2-months post-weight parity. Few group differences in adipose tissue secondary and tertiary bile acids were observed. The HF group had lower UDCA and ω-MCA concentrations than the LF and SW groups, respectively ($p<0.05$). The SW group had lower concentrations of DCA than the LF group. Concentrations of bile acids in muscle tissue were highly variable and no differences were detected. Plasma analyses will also be reported. Thus, we have identified bile acids whose concentrations return to lean-associated levels following weight loss, while several secondary bile acid conjugates in the liver and a secondary bile acid in the adipose did not. This may suggest that while bile acid metabolism in the liver may be reversed following weight loss, alterations in the intestinal metabolism of some bile acids may be affected long-term. In summary, bile acid profiles appear to be impacted by weight gain and loss, in a tissue specific manner. However, the true underlying reasons for these alterations and long-term health implications require further investigation. Bile acid metabolism is reversibly and, in some cases, irreversibly altered with weight gain/weight loss.

POSTER 249

Altered Nucleoside Metabolism in Diabetic Nephropathy

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Our recent work demonstrated that plasma nucleosides pseudouridine (PU) and dimethyl guanosine (DMG) predict type 2 diabetic nephropathy (DN) progression, but the mechanism underlying this increase is not well-understood. In order to study the metabolism and flux of the nucleosides in vivo systematically, we developed a targeted quantitative analysis of nucleosides by liquid chromatography/ tandem mass spectrometry (LC/MS). Mice which exhibit characteristic pathological features of DN (BKS db/db) and control (BKS db+) were used to examine the static and dynamic changes in nucleoside metabolism in DN. We performed in vivo metabolic flux analysis (MFA) by LC/MS following intraperitoneal administration of isotopically labeled substrates 13C Uracil (precursor of PU) and 13C hypoxanthine (precursor of DMG and allantoin) to 24-week diabetic and control mice. MFA revealed statistically significant elevations in label incorporation into PU and hypoxanthine (HX) in plasma with corresponding decrease in urinary labeling in diabetic mice compared with controls. In contrast, label incorporation of uracil, HX, PU, DMG and allantoin were unchanged in diabetic renal cortex, liver and muscle suggesting that renal and tissue metabolism was not the source of the altered plasma nucleosides. The elevated plasma levels and diminished urinary excretion strongly implicate altered tubular handling of nucleosides in DN. In order to determine if the altered nucleoside handling was specific to DN, we measured these altered nucleosides in baseline samples of subjects with stages 3 and 4 chronic kidney disease (CKD) from the CPROBE cohort (16 DN and 24 non-diabetic subjects). After adjusting for serum creatinine, plasma PU, DMG and allantoin, and urinary allantoin and HX were significantly associated with DN. Levels of plasma PU and DMG predicted renal progression in one year (defined as 20% decrease in eGFR or 1.5 fold increase in proteinuria) only in DN, but not in non-diabetic CKD. These results highlight the unique role for altered nucleoside metabolism and handling in the pathogenesis of DN using MFA

POSTER 250

First trimester plasma concentrations of amino acids and acylcarnitines as early biomarkers of gestational diabetes

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Gestational diabetes (GD) affects 5 - 10 % of pregnancies in Canada. GD have major consequences for both mother and fetus if not properly treated. Early care could minimize complications related to GD. Amino acids (AA) and acylcarnitines (AC) have been identified as potential biomarkers for predicting GD. However, few studies have focused on the early screening and diagnosis of GD before the usual window of 24 to 28 weeks of pregnancy. We conducted a pilot metabolomic study targeting AA and AC as first trimester biomarkers of GD risk. In this nested case-study, 50 women who developed GD were matched to 50 control according to maternal age, gestational age at blood sampling and body mass index (BMI). All participants were recruited within the institutions of the CHU de Québec between 2005 and 2010. Non-fasting plasma samples collected between 7 and 15 weeks of pregnancy were analyzed using our hybrid targeted/untargeted metabolomics platform developed on a UPLC-ESI-QTOF instrument. Targeted AA and AC were quantified using the isotope dilution technique and differences between mean concentrations were tested using ANCOVA with adjustment for matching variables. Untargeted metabolomics will also be performed to identify new potential biomarkers of GD. As expected, GD cases and controls had similar mean age (31.1 years), gestational age (11.8 weeks) and BMI (25.7 kg/m2). Out of the 8 AA and 8 AC that were quantified, mean plasma concentrations [95 % confidence interval] were significantly higher in the GD group compared to the control group for isobutyrylcarnitine (22.7 ng/mL [20.1-25.2] vs 17.9 µg/mL [15.4-20.5], p = 0.01) and leucine (15.2 µg/mL [13.9-16.6] vs 13.1 µg/mL [11.8-14.4], p = 0.02). Borderline significant differences were noted for isoleucine (7.74 µg/mL [7.11-8.37] vs

6.97 µg/mL [6.34-7.60], p = 0.08), tyrosine (10.4 µg/mL [9.85-11.0] vs 9.64 ug/mL [9.06-10.2], p = 0.06) and valine (23.1 µg/mL [21.8-24.5] vs 21.2 µg/mL [19.9-22.6], p = 0.05). Results indicate that first trimester levels of some AA and AC are elevated in women developing GD.

POSTER 251

The association of endothelial nitric oxide synthase gene G894T polymorphism and endothelial dysfunction markers in Russian patients with metabolic syndrome

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Metabolic syndrome contributes to the development of endothelial dysfunction that may be defined as inadequate the formation in the endothelium of various biologically active substances such as nitric oxide. The vascular damages are also mediated by cell adhesion molecules, such as vascular cell adhesion molecule-1 (vCAM-1) and intercelluar cell adhesion molesule-1 (iCAM-1), which are produced in response to effect of certain inflammatory cytokines.The association between endothelial nitric oxide synthase (eNOS) polymorphisms were studied in different ethnic populations. The results, however, were contradictory. We aimed to analyze the frequency of endothelial nitric oxide gene G894T polymorphism patients with metabolic syndrome from Russia and to evaluate its association with serum nitrite, iCAM-1, vCAM-1 levels. The study population consisted of 172 patients with MS(66 men and 106 women with a mean age of 43.9 ± 9.1 years and mean body mass index (BMI) 37.2 ± 5.6. kg/m2. The control group included 144 healthy donors (60 men and 84 women with a mean age of 36.9 ± 8.7 years and normal BMI (22,9 ± 2 kg/m2). The diagnosis of metabolic syndrome was defined according to the International Diabetes Federation criteria. All subjects were Caucasians and gave informed consent. Serum nitrite levels were measured after a colorimetric reaction with Griess reagents. Serum concentrations of iCAM-1 and vCAM-1 were determined by ELISA. The obtained frequencies of the eNOS G894T genotypes for the patients with metabolic syndrome were GG (44.2%), GT (46.5%), and TT (9.3%). The allele distributions of G and T were 67.4% and 32.6%, respectively. In the control group, the genotype frequencies were 61.8% for GG, 31.9% for GT, and 6.3% for TT, and the frequencies of the G and T alleles were 77.8% and 22.2%, respectively. The allele frequencies and genotype distributions in patient group were significant different from those of the controls (p = 0.004 and 0.008, respectively). Compared with the GG genotype, the GT and TT genotype could significantly increase the risk of developing metabolic syndrome (OR = 1.85, 95% CI = 1.17–2.94 and OR = 1.54, respectively CI = 0.66 – 3.59). There were no significant differences in between serum levels on nitrites, iCAM-1 and vCAM-1 between the patients and the control subjects. Patients with TT genotype had significantly higher vCAM-1 levels when compared to healthy donors with the same genotype (p=0.03). The effect of eNOS G894T polymorphism on the risk of metabolic syndrome in Russian population has not been explored yet.

POSTER 252

The Korean Metabolomics Study: study design and preliminary results on serum metabolic profiles for newly-developing diabetes mellitus

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A prospective cohort study was designed to identify metabolomic risk factors, associated with the conversion into a patient group of newly-developing metabolic syndrome and related disease from normal

population group. Objective of this study was to develop a prediction model of metabolic syndrome and related disease that including both metabolic traits and genetic polymorphisms, in addition to known traditional risk factors, and evaluate its effectiveness. Among the entire 159,844 participants who had voluntarily undergone health examinations between 1994 and 2013, 77,970 Koreans with more than three times of visiting record were selected at the first stage. For the next stage, 20,698 Koreans with more than three times of visiting record and blood samples of each visiting with informed consent were selected. As a preliminary investigation, a non-targeted metabolomics approach based on liquid-chromatography/mass spectrometry (LC/MS) was used to generate comprehensive metabolomic profiles from 60 serum samples of final study subjects (n=20), obtained from consecutive three visits. To the comparison between newly-developing diabetes group and non-diabetes group at each visit, a qualitative analysis was done with PCA (Principal Component Analysis) method. Participants were largely lower-middle aged at enrolment (mean age of 41.8 in men and 39.9 in women). Mean levels of FBS (91.1, 93.6, and 94.1 at first, second, and third visit in men; 85.7, 87.1, and 87.4 at first, second, and third visit in women) showed significant gradual increases throughout the same three visits. Among them, 20 Korean men between the ages of 40 and 49 years at enrolment who showed the conversion of diabetic status during the three times of visiting period were selected as a final dataset; they were consisted of 5 diabetic patients and 15 non-diabetic controls at baseline, followed by occurrence of 5 newly-developing cases among non-diabetic control group in each of two subsequent visit. Our study revealed significant metabolic variation on score plot in newly-developed diabetic individuals that are distinct from non-diabetic people. The compounds that had significantly altered levels in newly-developed diabetic individuals were Palmitoylcarnitine, 400.34 m/z, [M+H]+; LysoPC, 566.32 m/z, [M+Na]+; PC, 566.32 m/z, [M+Na]+; PS, 568.32 m/z, [M+H]+; PI, 591.33 m/z, [M+H-2H2O]+; Betaine aldehyde, 84.08 m/z, [M+H-H2O]+; Sarcosine, 90.06 m/z, [M+H]+; Phenylacetic acid, 165.06 m/z, [M+H]+; 9-hydroxy-decanoic acid, 171.14 m/z, [M+H-H2O]+; D-Tryptophan, 277.08 m/z, [M+Na]+; methyl 8-[3,5-epidioxy-2-(3-hydroperoxy-1-pentenyl)-cyclopentyl]-octanoate, 397.26 m/z, [M+H]+; α-Tocotrienol, 407.33 m/z, [M+H-H2O]+; Naringenin-4'-O-β-D-Glucuronide, 413.09 m/z, [M+H-2H2O]+; and Prostaglandin D2 serinol amide, 426.28 m/z, [M+H]+. We confirmed significant metabolites for newly-developing diabetes in Asian, and a prediction model including such novel biomarkers can be developed.

POSTER 253

Metabolomic analysis of clinical plasma from cerebral infarction patients presenting with blood stasis pattern

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Blood stasis (BS) pattern is characterized as a disorder of blood circulation. In traditional Korean medicine (TKM), it is viewed as a cause factor of diseases related with vascular disorders, such as multiple sclerosis, coronary heart disease and stroke. In this study, we investigated differences in plasma metabolomic profiles from subjects with cerebral infarction (CI) displaying BS or non-BS patterns. Thirty-one patients with cerebral infarction diagnosed with BS pattern and an equal number of sex- and age-matched non-BS patients were enrolled. Anthropometric parameters and symptom/sign index were measured. Metabolic profiling was performed using ultrahigh-performance liquid chromatography-quadrupole time-of-flight mass spectrometry and identification of each metabolites was confirmed from Human Metabolome Database. According to Korean Standard PI for Stroke, we investigated the distribution of 11 diagnostic symptoms of BS from patients with CI. Forty-one percent of the BS patients displayed purple tongue coloration (p=0.001), and some patients in the BS group had red or black spots in the surface of the tongue, which were not observed in the non-BS group (p=0.039). In addition, the ratio of subjects with rough pulse in the BS group was sighificantly higher than that of subjects in the non-BS group (p<0.001). OPLS-DA score plot showed different metabolic patterns between BS and non-BS group (P<0.0001). UPLC-MS data allowed us to find 82 metabolites that differed significantly between the BS and non-BS groups (p<0.05), and seven of them were identified. Levels of acyl form of carnitine

were significantly higher in the BS pattern than the non-BS pattern. Kynurenine, which is produced by oxidation of tryptophan, was increased 2.2 fold in the BS group (p=0.010). Another metabolites, creatinine, leucine and phosphocholine was also increased 1.3-1.6 fold in the BS group. Our results showed that some of metabolites, including acyl-carnitines, related with BS pattern in TKM

POSTER 254

Cardiovascular health revealed as correlation patterns and predictive relationships between serum lipoprotein and fatty acid features in non-obese Norwegian adults

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The favorable impact of diets rich in omega-3 fatty acid on the risk of developing cardiovascular diseases (CVDs), was suggested by the pioneering work of Dyerberg et al. (1975). They compared lipid levels in blood from Greenlandic Eskimos (with low incidence of CVD) with Eskimos living in Denmark with similar occurrence of CVD as ethnic Danes. We collected serum samples from non-obese healthy men and women and mapped their lipoprotein and fatty acid profiles with the aim to assess the degree of association between the profiles. Our work is based on the reasonable assumption that the fatty acid profile in serum reflects long term effect of dietary habits. Multivariate data analysis was used to reveal correlation patterns. 136 healthy volunteers, 69 women and 67 men that were ethnic Norwegian, were recruited among the inhabitants of a rural community in the Fjord region of Western Norway. Blood samples were collected between 8 and 9 am after overnight fasting. Serum was obtained according to a standardized protocol.Fatty acids were quantified on a gas chromatograph by a standardized procedure involving addition of an internal standard, esterification, identification of fatty acids using a template of retention time indices, and, chromatographic area corrections by empirical response factors. Serum lipoprotein subclasses were obtained on an HPLC system at Skylight Biotech (Akita, Japan) according to a standardized procedure. Preprocessing and multivariate data analysis was performed using the Sirius software. We have assessed the associations between lipoproteins and fatty acids in serum for a normolipidemic population of ethnic Norwegians living in a rural community in the Fjord region of Norway. Multivariate data analysis was used to model the relation between lipoprotein features and fatty acid profiles. Our assumption is that the fatty acid profiles in serum mirror dietary habits in the examined population and that diet will imprint its signature on the lipoprotein features. Genes, physical activity and other factors possibly influencing lipoprotein features represent confounding factors in our investigation, but since we have restricted our investigation to subjects of ethnic Norwegian from one region, diet is expected to be a dominant factor influencing the fatty acid levels and lipoproteins. Strong predictive associations were revealed for many lipoprotein features related to risk for development of cardiovascular diseases as well as for features implying good cardiovascular health. Eicasapentaenoic acid (EPA) and the ratio of EPA to arachidonic acid (AA) was found to have a stronger positive association to average size of high density lipoprotein (HDL) particles than docosahexaenoic (DHA) for both men and women and for average size of low density lipoprotein (LDL) particles in men. Concentration of HDL in both men and women correlated to EPA, but for women DHA correlated just as strongly to HDL concentration as EPA. For men docosapentaenoic (DPA) showed stronger association to HDL concentration than EPA. In conclusion, high ratio of EPA/AA seems to be the strongest biomarker for good cardiovascular health in both men and women. We believe that our approach based on molecular profiling of serum and multivariate analysis represents a useful alternative to self-reporting questionnaires and dietary interventions. Questionnaires have low resolution and dietary interventions are costly and difficult to carry out under controlled conditions over long time periods. Connect individual fatty acids to lipoprotein features by multivariate correlation and regression analysis to define patterns of cardiovascular health

POSTER 255

Correlation of Urinary Metabolites with Radiographic Progression of Knee Osteoarthritis in Overweight and Obese Adults

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Osteoarthritis (OA), the most common form of arthritis, is also the number one cause of disability in adults. Interventions which can modify the progression of OA are badly needed. Age, joint injury, and obesity are among the multiple risk factors for OA. The mechanism by which altered metabolism in certain obese individuals might contribute to OA pathogenesis is poorly understood. A NMR metabolomics analysis was conducted to determine if differences in metabolic profiles could be used to distinguish people with knee OA who exhibited radiographic progression, during an 18 month exercise and weight loss intervention, from those who did not. Urine samples collected from overweight or obese participants in the Intensive Diet and Exercise for Arthritis (IDEA) trial were selected. IDEA was a prospective, single-blind, randomized controlled trial that enrolled overweight and obese, older adults with knee pain and radiographic evidence of tibiofemoral osteoarthritis. Participants were randomized to one of three 18-month interventions (intensive dietary weight loss-only; intensive dietary weight loss-plus-exercise; or exercise-only control). OA progression was determined by using standardized knee radiographs. NMR metabolomics used to identify bins that differentiated progressors (decrease in JSW of ≥ 0.7mm) and non-progressors (decrease in JSW of ≤0.35mm), and the metabolites were identified using library matching. The progressors (P, n=22) and non-progressors (NP, n=22) were matched such that there were no differences in age (P, 66.5±5.3; NP, 66.4±5.3), BMI (P, 31.9±4.6; NP, 33.2±3.8), sex (15 females and 7 males in each group), or race (P, 18 Caucasians and 4 African Americans; NP, 19 Caucasians and 3 African Americans). NMR metabolomics analysis using urine could distinguish progressors and non-progressors of OA at both baseline and follow up (18 months). Multivariate data analysis (OPLS-DA) indicated that glycolate, hippurate, and trigonelline were among the metabolites that were important for distinguishing progressors from non-progressors at baseline whereas alanine, N,N-dimethyglycine, glycolate, hippurate, histidine, and trigonelline, were among the metabolites that were important for the discrimination of progressors from non-progressors at the 18 month follow-up visit. OPLS regression showed that the metabolomics data and the change in joint space width from baseline to 18 months was highly correlated for progressors (R2=0.9, Q2=0.3) as well as for non-progressors (R2=0.6, Q2=0.2). The results from these analyses suggest that metabolomics may be used to predict OA progression at baseline using non-invasive urine samples. Metabolic differences were found for the first time to correlate with radiographic progression in overweight and obese adults with knee-OA.

POSTER 256

Metabolomic Profile of Term Human Placenta: Effect of Obesity

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In all eutherian mammals the placenta plays an indispensable role in supporting embryonic and fetal development. As the only fetal tissue in direct contact with the maternal environment, the placenta mediates changes to the in utero milieu by regulating nutrient transport to the fetus. In the present study we employed a non targeted metabolomic approach to examine broad changes in placental pathways associated with maternal obesity. Term placenta from 30 normal weight (pre-pregnancy BMI between 19 - 25 Kg/m2) and 30 obese (pre-pregnancy BMI≥30 Kg/m2) women were collected at delivery as part of a

longitudinal observational study of mothers and infants. Placenta from both male and female infants and varying modes of delivery were included in this analysis. For metabolomics analyses samples were deproteinized with methanol and reconstituted in acetonitrile/water (1:1). Full MS scan in ESI positive and negative (m/z 60-900) modes and MS/MS data were acquired on a Thermo Scientific Q Exactive Orbitrap. LC/MS data was processed using Thermo SIEVE 2.1. Statistical results and putative IDs were generated through Simca-P and MetaboAnalyst 3.0. Using SIEVE 2.1, we extracted more than 7000 features (mass and retention time) in positive mode, and 5000 features in negative mode. PCA analysis demonstrated a clear distinction between groups (Normal Weight vs. Obese) in both ionization modes. Multivariate analysis of these features resulted in over 100 discriminant metabolites in positive and in negative mode. Of these metabolites, cysteine ($p < 0.001$), GSSG ($p < 0.001$), and GSH ($p < 0.001$) have been confirmed. Pathway analysis links these metabolites to changes in glutathione metabolism between normal weight and obese women, which is consistent with the reported role of oxidative stress in obese pregnant women. Taurine ($p < 0.05$) was also confirmed to have higher concentrations in placenta from normal weight women, which suggests a reduction in placental taurine transporter activity in obese pregnancies, as previously reported in the literature. This study highlights the potential of applying metabolomics methods using term placenta for understanding maternal obesity effects on placental function

POSTER 257

Characterizing Novel Metabolic Syndrome Phenotypes at Risk for Coronary Artery Disease with HDL Proteomic and Glycomic Profiling

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Coronary artery disease (CAD) is the cause for one in four deaths in the US according to the CDC, accounting for ~600,000 deaths each year. Despite the fact that HDL cholesterol (HDL-C) is protective against CAD, pharmaceutical interventions to increase HDL-C have failed to demonstrate benefit in cardiovascular endpoints, albeit resulting in increased HDL-C levels. It is quite likely that in addition to HDL-C levels, the glycomic and proteomic profiles of HDL surface proteins also play a significant role in CAD. Our primary aim was to determine whether combined analysis of HDL proteome and glycome can distinguish individuals with coronary artery disease (CAD) from those without. Our study included 10 patients with CAD and 10 patients without CAD, with five females and five males in each group. Of the 20 subjects, 10 selected CAD patients had a higher arterial score than non-CAD subjects. Plasma samples from these subjects were used to isolate HDL using sequential flotation ultracentrifugation. A shotgun proteomics technique using trypsin digestion and reverse phase LC-MS/MS was used to analyze HDL associated proteins. X! Tandem was used to achieve accurate identification of the proteins. A parallel method was used to determine the glycosylation of the isolated HDL by first cleaving the N- and O-glycans followed by analysis with nano-HPLC-Chip QTOF MS. Data from these were analyzed using univariate and multivariate statistics. The CAD subjects were older (57.2 vs. 50.3y, $p = 0.03$), and had higher fasting plasma triglycerides (195.3 vs. 146.2 mg/dL, $p = 0.021$), but had lower BMI (26.3, vs. 30.6 kg/m2, $p = 0.001$) compared to the non-CAD subjects. Principal components analysis showed a clear separation of the CAD and non-CAD subjects, confirming that combined HDL proteomic and glycomic profiles distinguished at-risk subjects with atherosclerosis from those without. The HDL proteome, especially the apolipoprotein profile (specifically Apo AI, AII and E, $p < 0.05$), showed differences between the CAD and non-CAD subjects, with the CAD subjects consistently having less apolipoproteins than the non-CAD subjects. In addition, CAD patients had lower HDL apolipoprotein content, and lower serum amyloid A2 (SAA2, $p = 0.020$) and SAA4 ($p = 0.007$) but contrastingly higher sialylated glycans ($p < 0.05$). Amidst individuals equally at risk for CAD, integrated HDL proteomic and glycomic profile may reveal

novel biomarkers indicating elevated risk.

POSTER 258

Lipidomics of plasma lipoprotein fractions in myocardial infarction-prone rabbits

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Lipids play important roles in the body and are transported to various tissues via lipoproteins. Lipoproteins consist of a core of hydrophobic lipids covered by a layer of apoproteins and phospholipids, which allows them to be transported in the aqueous environment of blood. It is commonly assumed that alteration of lipid levels in lipoproteins leads to dyslipidemia and serious diseases such as coronary artery disease (CAD). However, lipid compositions in each lipoprotein fraction induced by lipoprotein metabolism are poorly understood. Lipidomics is expected to provide valuable information regarding the pathogenic mechanism of CAD. Here, we performed a lipidomics of plasma and its lipoprotein fractions in myocardial infarction-prone Watanabe heritable hyperlipidemic (WHHLMI) rabbits. Three Japanese white (JW) rabbits (23 months old, Kitayama Labes, Co. Ltd., Ina, Japan) and five WHHLMI rabbits (21 months old) were used. Plasma lipoproteins were fractionated by ultracentrifugation with a stepwise method. Lipid extraction from rabbit plasma, LDL, and VLDL was performed using Bligh and Dyer's method. Lipid molecular species were analyzed using supercritical fluid chromatography hybrid quadrupole-Orbitrap mass spectrometry (SFC/Q-Orbitrap MS). Various lipids were identified according to the accurate information for the precursor ion (MS) and its representative product ions (MS/MS) in the positive-ion mode and in the negative-ion mode. The difference of lipid levels was evaluated based on the peak area using Student's t-test. In total, 172 lipids in plasma obtained from normal and WHHLMI rabbits were quantified with high throughput and accuracy using SFC/Q-Orbitrap MS. Plasma levels of each lipid class (i.e., phosphatidylcholine, phosphatidylethanolamine, phosphatidylinositol, lysophosphatidylcholine, lysophosphatidylethanolamine, sphingomyelin, ceramide, triacylglycerol, diacylglycerol, and cholesterol ester, except for free fatty acids) in 21-month-old WHHLMI rabbits were significantly higher than those in JW rabbits. High levels of functional lipids, such as alkyl-phosphatidylcholines, phospholipids including ω-6 fatty acids, and plasmalogens, were also observed in WHHLMI rabbit plasma. In addition, high-resolution lipidomics using very low density lipoprotein (VLDL) and low density lipoprotein (LDL) provided information on the specific molecular species of lipids in each lipoprotein fraction. In particular, higher levels of phosphatidylethanolamine plasmalogens were detected in LDL than in VLDL. Our lipidomics approach for plasma lipoprotein fractions will be useful for in-depth studies on the pathogenesis of CAD. We identified potential lipid biomarkers of CAD from plasma lipoprotein fractions of myocardial infarction-prone rabbits.

POSTER 259

Probing metabolic perturbations in human heart failure: Spotlight on circulating acylcarnitines analysis by LC-QQQ

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The application of metabolomics to the clinic has revealed distinct metabolic patterns in patients with cardiovascular diseases such as myocardial ischemia/reperfusion or coronary artery diseases. Perturbations in the metabolism of energy nutrients, particularly of fatty acids (FA), have also been reported in heart failure (HF) patients, but data interpretation is often confounded by factors such as multi-medications, etiology/comorbidities and biological traits such as age and sex. Thereby, the aim of this exploratory study was to test the hypothesis that despite being optimally treated, HF patients will display altered circulating levels of energy-related metabolites, particularly those reflecting dysregulation of FA oxidation, namely acylcarnitines (ACs), which will persist after correction for age and sex and will be consistent across etiology/comorbidities. Circulating levels of 33 metabolites were assessed in plasma of 72 healthy and 68 ambulatory, asymptomatic, and optimally treated HF subjects along with clinically- and biochemically-relevant parameters. These included: (i) 9 Krebs cycle-related metabolites and 12 amino acids (by isotope dilution gas chromatography-mass spectrometry (GC-MS)), as well as (ii) 12 ACs (using a routine shotgun targeted electron impact (EI)-MS approach). Further to our finding of negligible MS signals for hydroxy-ACs, dicarboxylic-ACs and most unsaturated ACs, we developed a semi-quantitative LC-QQQ method, which enabled the detection of > 50 AC species and applied to a subset of the healthy and HF subject cohort as well as to a 2nd independent cohort of 8 healthy and 8 HF subjects. HF patients from the 1st cohort were in NYHA classes II and III, had a markedly depressed left ventricular ejection fraction (25.9 ± 6.9%) and were heterogeneous in term of etiology and comorbidity. Compared to controls, they were slightly older (66 ± 10 vs. 59 ± 9 years; p |0.2|). Of all the metabolites measured, 9 of them, which were all ACs and included saturated long chain ACs (LCACs) and acetylcarnitine (C2), showed a score similar to BNP and urea. These 9 ACs were significantly increased in HF patients after adjustment for age, sex, HOMA-IR and eGFR using an analysis of covariance ($p<0.0001$) and were also positively correlated with BNP ($p<0.01$). The observed pattern was consistent across etiology/co-morbidity. Using LC-QQQ, we detected an increase in several other AC species beyond saturated LCACs in HF patients from the 1st cohort, namely unsaturated ACs, hydroxy-ACs and dicarboxylic ACs. Similar results were observed for the HF patients from the 2nd cohort, thereby reinforcing our findings. Taken altogether, our finding of increased circulating ACs in HF patients highlights major lipid metabolic perturbations, which are independent of sex and age. Furthermore, the great diversity in these AC species suggests that they are perturbations in various intracellular FA metabolizing pathways. Future study should investigate the utility of these ACs as circulating marker to monitor disease severity or response to interventions in HF patients. Circulating ACs in human HF represent potential biomarkers of FA metabolism dysregulation.

POSTER 260

Using a metabolomics approach to explore effects of glucose, insulin and potassium treatment on reducing adverse outcomes in cardiac surgery

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Aortic stenosis is a common cardiac condition which can result in chest pain, fainting and congestive heart failure. A consequence of aortic stenosis is left ventricular hypertrophy (LVH) which is an independent predictor of poor patient outcome. Surgical treatment with aortic valve replacement in patients with both aortic stenosis and left ventricular hypertrophy has a mortality rate of around 3%. Clinical trials have demonstrated the protective effect of glucose, insulin, potassium (GIK) treatment during cardiac ischaemia. It is unclear whether the effects are due to mitigating the damage due to ischaemia or whether it improves the recovery post ischaemia. In order to answer this question, we used a metabolomics approach to attempt to discover how GIK treatment exerts its effects. Patients undergoing aortic valve replacement surgery for aortic stenosis with LVH were treated with either a continuous infusion of GIK or a placebo control solution of 10% dextrose from sternotomy until 6 hours post ischaemic insult. Cardiac biopsies were taken at three time points during surgery – before ischaemic insult, at the maximum ischaemic insult, and ten minutes post ischaemia. Biopsies were extracted using a

two-step biphasic method of chloroform, methanol and water and analysed using direct-infusion mass spectrometry with a Thermo LTQ-FT ultra. Data was processed according to the SIMstitch method. Missing values were filled in using a K-nearest neighbour approach before normalisation using a probabilistic quotient normalisation technique and transformation using a generalised log transformation before statistical analysis. Data were analysed using a combination of univariate and multivariate statistical methods. Initial analysis using Principal Components Analysis (PCA) and Partial Least Squares Discriminant Analysis (PLS-DA) showed a difference due to the biopsy time point but not due to treatment. Individual variation was a large confounder in the analysis. To maximize the design of study, ANOVA-Simultaneous Components Analysis (ASCA) was also used to evaluate the data. ASCA enables the metabolic changes due to recovery to be separated from the metabolic changes due to the treatments' effect on recovery. The majority of the variation due to the effect of GIK treatment (71% of the variation) was seen to occur in the period between no ischaemic insult and maximal ischaemic insult, indicating that the effects of the GIK treatment were primarily on preventing ischaemic damage. A smaller effect was seen in the time point post recovery suggesting that GIK may also hasten recovery following ischaemic damage. The metabolites that were contributing most to the treatment part of the model were putatively annotated and mapped to known metabolic pathways. Metabolic changes included an increase in glutamine and phosphoenolpyruvate and a decrease in aspartate and α-ketoglutarate among others. The results strongly suggested that one of the effects of GIK treatment, with respect to changes seen in the control patients over the same time period, is to change energy regulation during periods of ischaemia by affecting the TCA cycle. Mass spectrometry metabolomics and ASCA are used in a clinical trial to determine a possible mechanism for GIK treatment.

POSTER 261

Non-targeted metabolomic profiling of plasma identifies novel metabolic markers associated with cardiovascular events

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Cardiovascular disease (CVD) is the leading cause of mortality in the US and worldwide. Successful treatment of CVD requires advanced mechanistic understanding of the disease process and accurate risk stratification. Our understanding of the molecular physiology of CVD is incomplete, and current clinical models for cardiovascular risk prediction are insufficient. Metabolomic profiling has shown potential in discovering novel biomarkers and improving the risk stratification of CVD. Previous metabolomic studies have identified novel biomarkers in coronary artery disease (CAD) and congestive heart failure. In this study, we performed non-targeted metabolomic profiling of 78 patients enrolled in the Duke CATHGEN biorepository, which consists of sequential patients referred for cardiac catheterization for concern of ischemic heart disease from 2001 – 2011. The study cohort consisted of 38 patients undergoing cardiac catheterization who subsequently experienced cardiovascular events (death, myocardial infarction or percutaneous coronary intervention) over the ensuing two-year period and an age, gender, race and CAD index-matched control group of 40 event-free patients. Non-targeted metabolomic assay was performed using GC-MS. GC-MS data were deconvoluted with AMDIS freeware and alignment was performed by parsing the spectra against peaks annotated by the Fiehn libraries with additions from the metabolomics laboratory at Duke. Each sample point consisted of the log-2 transform of the integrated peak areas. Statistical analysis included univariate tests (Fisher's exact test and Wilcoxon ranked sum test), LASSO regression and exploratory analysis using principal component analysis and Bayesian sparse latent factor models. The median age of the cohort was 65 years (range 44 – 86) and 53% (n = 41) of patients were male. Baseline medical conditions were similar between the event and control groups, including medical history (hypertension, diabetes mellitus, dyslipidemia, smoking history, prior coronary artery bypass grafting, New York Heart Association functional classification), family history of CAD, laboratory tests (serum creatinine, glucose, glycated albumin and cystatin), number of diseased coronary arteries and left

ventricular ejection fraction. A total of 208 metabolites were identified by GC-MS and annotated. Univariate analysis revealed statistically significant elevation of metabolites in the pentose phosphate pathway in the event group compared to controls. Specifically, C4 and C5 sugars (erythronic/threonic acid, aldopentose) and sugar alcohols (erythritol/threitol and ribitol) differentiated events from controls (median difference = 0.5, 0.5, 0.4 and 0.45; p = 0.003, 0.01, 0.005 and 0.02). Decanoic acid, 2-hydroxyglutaric acid, an endogenous carcinogen and pseudouridine, a marker of oxidative stress and RNA damage, were also significantly elevated in the event group compared to controls (median difference = 0.5, 0.49 and 0.54; p = 0.02, 0.02 and 0.01). LASSO regression using cardiovascular events as the outcome variable and all identified metabolites as covariates selected erythronic acid/threonic acid, erythritol/threitol, aldopentose and 2-hydroxyglutaric acid as the main features predictive of future cardiovascular events. Multivariate analysis using principal component analysis and Bayesian sparse latent factor models discovered multiple latent factors corresponding to highly correlated metabolite groups. Logistic regression using these factors revealed that the factor containing C4 and C5 sugars and sugar alcohols was positively associated with cardiovascular events. This study performed non-targeted metabolomic profiling of CVD events and discovered novel biomarkers associated with CVD events.

POSTER 262

Predictive cardiovascular metabolomics: heart failure and the response to therapy

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Heart failure (HF) is associated with ventricular dyssynchrony and energetic inefficiency, which can be alleviated by cardiac resynchronization therapy (CRT). The aim of this study was to determine the metabolomic signature in HF and its prognostic value for the response to CRT. This prospective study consisted of 24 patients undergoing CRT for advanced HF and 10 control patients who underwent catheter ablation for supraventricular arrhythmia but not CRT. Blood samples were collected before and 3 months after CRT. Metabolomic profiling of plasma samples was performed using GC/MS and 1H NMR. The plasma metabolomic profile was altered in the HF patients, with a distinct panel of metabolites, including Krebs cycle and lipid, amino acid, and nucleotide metabolism. CRT improved the metabolic profile manifested in increased succinate/glutamate and glucose/palmitate ratios, indicators of Krebs cycle, glycolytic and fatty acid metabolism. The responders to CRT had a distinct baseline plasma metabolomic profile, including higher isoleucine, phenylalanine, leucine, glucose, and valine levels and lower glutamate levels. In nonresponders, higher plasma levels of citrate, creatine and creatinine after CRT indicate persistent energetic deficiency. Thus, CRT improves plasma metabolomic profile of HF patients indicating harmonization of myocardial energy substrate metabolism. A prognostic panel of metabolites has been established which correlate with heart LVEF in HF pre- and post-CRT and discriminate responders from nonresponders to CRT. This study determined the metabolomic signature in patients with HF and its prognostic value for the response to CRT.

POSTER 263

Global metabolic profiling reveals associations of serum metabolome with gut microbiome and insulin sensitivity

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Insulin resistance (IR) and the metabolic syndrome (MetS) are established risk factors for both type 2 diabetes (T2D) and cardiovascular disease. Here we describe the serum metabolomics study of 291 non-diabetic and 75 T2D individuals from Denmark. The metabolic signatures were evaluated together with the IR and MetS phenotypes as well as gut microbiome gene repertoire obtained by deep shotgun sequencing of the faecal microbiome. A Leco Pegasus 4D GC×GC-TOFMS instrument was used for the analysis of polar metabolites. The columns were as follows: 10-m Rxi-5MS (i.d. 0.18 mm, df 0.18 µm) + 1.5 m BPX-50 (i.d. 0.1 mm, df 0.1 µm). The samples were derivatized with 25 µl MOX (45°C, 60 minutes) and 25 µl MSTFA (45°C, 60 minutes). Data were processed using the Guineu software. Molecular lipids were analysed on a Waters Q-Tof Premier mass spectrometer combined with an Acquity UPLC using an Acquity UPLCTM BEH C18 2.1 × 100 mm column with 1.7 µm particles. Data were processed using the MZmine 2 software. For the gut microbiota analysis, Illumina shotgun sequencing was applied to DNA extracted from faecal samples. For all 366 study-participants, serum metabolomic profiles were generated using GCxGC-TOFMS for the charactization of polar metabolites (94 known, 231 unknown) and UHPLC-QTOFMS for the characrerization molecular lipids (289 known, 587 unknown). The GCxGC-TOFMS analysis included quantitative analysis of selected key metabolites and semiquantiative profiling the other metabolites. The UHPLC-QTOFMS data was normalised with class-specific internal standards for the semiquantitative profiling of the molecular lipids. All metabolite peaks that were present in over 50% of samples were included in the data analyses, including the unidentified ones. The unidentified peaks were annotated with their structural class. Several metabolites that are of microbial origin were identified with the GCxGC-TOFMS, including several phenolic compounds such as indoxyl sulphate, hippuric acid and indole derivatives. The data analysis showed that several metabolite clusters were correlating with the insulin resistance and the gut microbiota. The identifiable metabolites constituting the IR-metabotype mainly included amino acids and tricarboxylic acid cycle metabolites, as well as a large number of triacylglycerols. Particularly, the branched-chain amino acids (valine, leucine, isoleucine) that were analysed quantitatively with the GCxGC-TOFMS, co-clustered with hydrocinnamic acid and indole-3-lactic acid, both from a class of phenolic compounds, which are associated with gut microbial metabolism. These findings link the composition of the gut microbiome, the serum metabolite levels and the metabolic phenotypes. Functional imbalances in the human gut microbiome are linked to serum metabolite levels relevant for the host metabolic health.

POSTER 264

Serum metabolites and gene expression profiling in peripheral white blood cells in response to the food-intake with different glycemic index

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It is important to prevent postprandial hyperglycemia, because it may contribute to the pathogenesis and development of type 2 diabetes and cardiovascular disease. We hypothesized that there were two patterns of postprandial blood glucose suppression with high and low insulin secretion. Metabolomics and transcriptomics technology in human nutrition intervention studies would allow for screening of the effects of nutrients. We investigated the selective effects of hyperglycemia and hyperinsulinemia on metabolite and gene expression in peripheral blood. In a randomized crossover study, 10 obesity subjects consumed test meals of 75g glucose (Glu), high-glycemic and high-insulin meal with potato (Con), low-glycemic and low-insulin meal with viscous meal (Vis), low-glycemic and high-insulin meal with whey of ricotta cheese (Che) and yogurt (Yog). Venous blood samples were collected at 0 min and at 30, 45, 60, 90, 120 and 240 min after meal intake and were used to analyze glucose, insulin and metabolite levels; the peripheral white blood cells samples taken at 0, 120, and 240 min were also used for microarray analysis, and those taken at 0, 60, 120, 240 and 360 min were used for real-time PCR. Serum valine, leucine and isoleucine

decreased after Glu intake, but increased after Con, Vis, Che and You. Serum pyruvate level after Vis intake was higher than other meals at 240 min. Serum lactate level after all meals increased and that in Che acutely decreased at 60 min. GLPOSTER 1 level increased after all meals intake, but there were not significant among different meals. GIP after Che intake significantly increased compared with Con, Vis or You intake at 45 and 60 min. Pyruvate dehydrogenase kinase, isozyme 4 (PDK4), carnitine palmitoyltransferase 1A (liver; CPT1A) and the solute carrier family 25 (carnitine/acylcarnitine translocase), membe 20 (SLC25A20) gene expression after all meals were significantly lower than at 0 min, respectively. However, there were not significant differences between each meal type. Different glycemic index food intake showed specific serum metabolites and gene expression patterns at postprandial periods.

POSTER 265

Metabolomics of cardiomyocytes

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Certain diseases like myocardial infarction and stroke cause massive cell death, which human body cannot repair. Adult brain and heart stem cells have potential to actively regenerate the tissue and produce new cells. However, these endogenous stem cell repair mechanisms are underpowered for the tissue repair in ischemic and traumatic diseases. A newborn mouse has the capability to regenerate cardiac muscle after cardiac injury, but the ability is lost within seven days. The aim of this study was to analyze the change in cardiac cell metabolism in mice during the early postnatal period. Metabolic profiles of mouse heart cells were analyzed at two time points to reveal changes in metabolites. Metabolites were studied using non-targeted approach and without prior information about possibly changing metabolites. For this purpose the cells were homogenized with ultrasound and proteins were precipitated with methanol. After centrifugation the cell extracts were analyzed with ultra-performance liquid chromatography-quadrupole time-of-flight mass spectrometry (UPLC-QTOF) using electrospray ionization in positive and negative ion mode. We had 10 pooled samples of mouse heart cells collected at one day after birth (P1) and 10 pooled samples of mouse heart cells at nine days after birth (P9). The cell amount was 50 000 cells in each sample. Principal component analysis of dataset in positive ion mode showed clear differences between metabolites in different sample groups. The same kind of distribution and grouping can be seen from clustering analysis of samples as well. In dataset several hundred significantly changed metabolites (with 1 % significance level) were seen with varying fold changes. The total protein content of sample group pools revealed that even though the cell numbers in samples was equal, protein content was doubled in the cell samples at P9. Metabolites were normalized with internal standard and cell number as well as taking into account of the protein amount. Data-analysis was done to both datasets; normalized to protein content as well as normalization to cell number. When both datasets were taken into account approximately 50 upregulated metabolites were detected in P1 samples with a fold change of at least 2 ($p \leq 0.002$). With the same selection criteria the number of downregulated species at P1 was approximately 20 metabolites. With the biggest fold changes and statistical difference there were a few unknown metabolites, which could only be detected in P1 samples. These metabolites as well as other uPoster or downregulated species will be investigated further and identified as far as possible. Metabolic changes related to the loss of regeneration capability of cardiomyocytes

POSTER 266

Podocyte-specific protein tyrosine phosphatase 1B deficiency in mice improves glucose homeostasis and proteinuria under diabetic mellitus condition

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Diabetic mellitus (DM) nephropathy is a serious problem that causes renal failure. Protein tyrosine phosphatase 1B (PTP1B) is a physiological regulator of glucose homeostasis. The metabolic role of PTP1B in insulin-responsive tissues has been reported but its function in the kidney remains largely unknown. In addition, a recent study demonstrates that insulin signaling in podocytes is critical for kidney function. Moreover, nephrin which is key podocyte protein is a PTP1B substarte. In the current study, we assessed the physiological role of PTP1B in podocytes using the Cre-loxP system to achieve podocyte-specific deletion performed biochemical and metabolomics studies to uncover underlying molecular mechanism. We generated control mice (CT) and podocyte-specific PTP1B knockout mice (KO) using Cre-loxP approach. In addition, control and KO mice were intraperitoneally injected with streptozotocin to generate DM model for each study group. Insulin tolerance tests (ITTs) and glucose tolerance tests (GTTs) were used to evaluate glucose homeostasis, and serum and urine components to determine kidney function. Further, we analyzed liver and kidney samples from control and KO mice using advanced LCMS methods for profiling steroids to gain insights into the molecular mechanism. No significant metabolic differences were observed in CT and KO mice under basal conditions. However, STZ-injected KO mice exhibited improved insulin sensitivity, as measured by ITT, compared with STZ controls in the absence of any body weights. In addition, STZ KO mice displayed improved fasted, fed blood glucose, serum creatinine, urine albumin levels and maintained serum albumin levels compared to STZ CT mice. At the molecular levels, podocyte cell lines with PTP1B knockdown exhibited enhanced insulin signaling compared with controls under normal and high glucose conditions. In addition, metabolomics analyses of kidneys from CT and KO mice (with and without STZ treatment) revealed increased bile acids (Chenodeoxycholic acid, Deoxycholic acid and Tauroursodeoxycholic acid) in STZ KO mice compared with controls. On the other hand, progesterone and corticosterone from liver samples tended to increase in STZ KO mice. These findings reveal that PTP1B in podocyte regulates systemic glucose homeostasis and kidney function.

POSTER 267

Application of LC-MS/MS based metabolomics for the study of acute coronary syndrome

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Acute coronary syndrome (ACS) is a leading cause of morbidity and mortality. The strong relationship between ACS and troponin I and T has been recognized for decades. However, beyond these well-established associations, there is a strong possibility that other links between metabolic dysregulation and ACS remain to be discovered. Metabolomic studies hold promise for the discovery of pathways linked to disease processes and recent advances in metabolomic technologies have enhanced the possibility of better understanding the knowledge of its physiopathology through the identification of the altered metabolites associated with different pathways. In this study, untargeted metabolomics approach was employed to reveal the metabolic differences between ACS and non-ACS patients. Peripheral plasma samples from 25 ST segment elevation ACS patients and non-ACS patients were collected before and 24 hours after the angiography. Liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–TOF MS) and orthogonal partial least squares (OPLS) permitted the identification of many metabolites with statistical differences ($p<0.05$) between experimental groups. Clear separation between ACS and Non-ACS were observed in OPLS-DA plot. Different phosphatidylcholines (PC), glycero-phosphatidylethanolamines (PE), carnitine, glycero-phosphatidic acids (PA), Clopidogrel carboxylic acids and carbohydrates were identified. This panel of biomarkers reflects the phosphatidylcholines metabolism on gut-flora are closely associated with pathogenesis of ACS that could be used for diagnosis of ACS. Promising biomarkers have been identified for disgnosis of ACS

POSTER 268

Urinary Metabolomic Profiling of Pulse-fed Spontaneously Hypertensive Rats (SHR): The Relationship to Hypertension

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Hypertension affects >50% of North Americans. While pharmacological agents are capable of reducing hypertension, it has been suggested that future hypertension development in persons with prehypertension may be controllable primarily through diet and lifestyle changes. Consumption of pulses (dried beans, peas, lentils and chickpeas) is associated with a reduced risk of hypertension. We have shown that lentils are the most effective pulses for attenuating the blood pressure rise that occurs in spontaneously hypertensive rats (SHR) as they age. The objective of the present study was to conduct metabolomic profiling of the pulses and the urine samples from the aforementioned study and determine which compounds in pulses and urine samples are related to blood pressure lowering in the lentil-fed SHR. Male SHR (n=8/group) were fed one of 5 semi-purified diets prepared with freeze-dried powder (30%, w/w) from cooked beans (mixture of pinto, red kidney, white navy, and black beans), peas (yellow and green), lentils (green and red), chickpeas, or mixed pulses (all of the aforementioned) or a control (non-pulse diet) for 4 weeks. Normotensive WKY rats (n=8/group) were fed either the mixed pulse or non-pulse diets for the same time period. During the final week of dietary intervention, urine samples were collected during a 12-hour fast in metabolic cages. The urine samples and pulse powders were extracted with acetonitrile prior to analysis with an Agilent 6538 LC-MS-QTOF. Data processing was performed using Agilent Mass Hunter Qualitiative and Mass Professional Profiler. SHR on a lentil-based diet experienced an increase in systolic blood pressure (SBP) of 1 mmHg and diastolic blood pressure (DBP) of 10 mmHg over the study period (4 weeks), compared to increases of 26 mmHg and 32 mmHg in SBP and DBP, respectively, experienced by the SHR control animals. In contrast, the other diets (beans, peas, chickpeas, and a mixture of all) did not significantly attenuate the increase in BP that occurred over the 4 weeks of the study. A moderated T-statistical test was performed between SHR fed control diet versus SHR fed the lentil diet to identify the urinary metabolites with significant differences ($P<0.01$). Several key metabolites associated with nitric oxide metabolism were significantly increased in urine samples from lentil-fed SHR, including citrulline and homocitrulline (involved in urea cycle and arginine metabolism), which were totally absent in SHR controls, as well as 2-oxoarginine, and some tripeptides. Several arginine-related compounds were detected in the pulses, of which Nω-hydroxyarginine, which has a potential role in nitric oxide formation, was detected only in green and red lentil extracts. These data suggest that metabolites affecting arginine metabolism may be linked to the lower blood pressure obtained in response to lentil consumption in SHR. A non-targeted urinary metabolomics approach applied to an SHR rat model in the present study was successful for monitoring hundreds of compounds and for the identification of the significant changes in metabolites after lentil consumption. These metabolites (eg. citrulline) provide evidence for a potential role of NO vasodilation in SHR rats that have eaten lentils. A differential analysis of arginine-related compounds detected in pulse powders enabled us to identify Nω-hydroxy-L-arginine as a key compound only present in green and red lentils and potentially responsible for the BP lowering capacity of lentils in the SHR model. An LC-MS based urinary metabolomics approach was successful in identifying select compounds in lentils with potential anti-hypertensive actions.

POSTER 269

Comparative Profiles of Cholesterol and Glycosphingolipids between Normotensive and Hypertensive States in DBA/2J Mouse Aqueous Humor

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To determine the profiles of cholesterol and glycosphingolipid (psychosines) species and their quantitative differences between normotensive and hypertensive intraocular pressure states derived from DBA/2J mouse aqueous humor (AH). Normotensive and hypertensive AH was sampled from mice by paracentesis. Lipid extraction was performed using suitable modifications of the Bligh and Dyer method. Protein concentration was estimated using the Bradford colorimetric assay. Cholesterol and

glycosphingolipids were identified and subjected to ratiometric quantification using appropriate class specific lipid standards on a TSQ Quantum Access Max triple quadrupole mass spectrometer. The comparative profiles of normotensive and hypertensive DBA/2J mouse AH showed several species of cholesterol and glycosphingolipids common between them. A number of unique lipids were also identified in normotensive AH that were absent in hypertensive AH and vice versa. A number of cholesterol and glycosphingolipid species were found to be uniquely present in normotensive, but absent in hypertensive AH and vice versa. Hypertensive AH also showed an overall decrease in levels of cholesterol and in levels of glycosphingolipids. Results will lead to further understanding the role endogenous lipids in the AH play in the pathologic state of glaucoma.

POSTER 270

Pharmacometabolomics Study: Reveals that Metformin Treatment Impacts the Urea Cycle

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Metformin is one of the most widely prescribed drugs for type 2 diabetes. Previous studies have shown that its anti-diabetic effect is mainly due to the activation of adenine monophosphate-activated protein kinase, AMPK. In addition to its anti-glycemic effects, metformin has been shown to have beneficial effects on the cardiovascular system, in polycystic ovary syndrome, and in the prevention of tumor recurrence. Here, we use metabolomic approaches to investigate the global changes of metabolites in plasma in response to metformin treatment in healthy volunteers. The significantly changed metabolites lead to new insights into metformin mechanism. Furthermore, these metabolic signatures may be used as early biomarkers of metformin response. 33 healthy African-American volunteers were admitted into the Clinical Research Services Facilities at San Francisco General Hospital for 3 days. Two oral doses of metformin (1000mg and 850mg) were given. Fasting baseline and two additional plasma samples at two times after metformin dosing were selected to measure metabolite levels using the GC-TOF platform. Metabolites in response to metformin exposure were analyzed using Wilcoxon paired t-test and the correlated direction was determined by the Spearman's rank correlation. Association of metabolites and metformin levels was analyzed by linear regression. The metabolic pathway analysis was based on the Human Metabolome Database (HMDB v3.5). Significantly changed metabolites were validated in mice intraperitionally dosed with metformin for 7 days. Compared to baseline, 39 metabolites were significantly changed at peak metformin concentrations in plasma. 26 were structurally identified. The top known metabolites were hypoxanthine, maltose, citrulline, ribose, tyrosine, and ornithine (q value<0.008). These metabolites were implicated in various pathways including the urea cycle, cysteine metabolism, amino-sugar metabolism, and glucose-alanine cycle. Interestingly, 5 metabolites overlapped in the urea cycle (25% enrichment). A significant enrichment of metabolites in the urea cycle, which represents the protein degradation cycle, is associated with metformin exposure. Two metabolites were significantly associated with glucose response to metformin. In particular, 2-hydroxybutanoic acid was correlated with glucose AUC after metformin treatment. These results suggest that metformin might have substantial effects on protein degradation pathways.

POSTER 271

Metformin: examining the effect of the drug using an un-targeted metabolomics method on a clinical study and HepG2 treated cells

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Type 2 diabetes is a complex metabolic disease associated with many risk factors including obesity, dyslipidemia, hypertension and smoking. Although there are many theories, the pathogenesis of type 2 diabetes is not well understood. Similarly, the mechanism of action of the first line drug for the majority of patients, metformin, is also not yet fully defined. Metabolomics is a holistic approach to the analysis of the small molecule components of biological systems. Using an untargeted approach on biological samples from a randomized control trial of metformin (CAMERA) and treated cell cultures may give a fresh insight into the metabolic mechanisms by which this disease and drug acts. Human plasma and HepG2 cell culture samples (treated with increasing concentrations of metformin) were extracted using a chloroform:methanol:water (1:3:1 ratio) extraction buffer. The samples were then analysed using a 22-minute un-targeted liquid chromatography-mass spectrometry metabolomics method. This was carried out on a Thermo Fisher Orbitrap Mass Spectrometer using a Merck SeQuant® ZIC®-pHILIC 5 µm, 150 x 4.6 mm column. Due to size, an in-house bespoke statistical analysis was carried out on the cohort study data, which included a batch normalisation algorithm and calculated the delta change over time of each metabolite. It was also run through IDEOM in batches to find trends along with the HepG2 cell data. Using the in-house bespoke statistical analysis with batch normalization and delta change calculation, preliminary results suggest that there are around 500 significantly different ($p < 0.05$) detected peaks of which roughly 130 of those being putatively identified metabolites. A large number of those metabolites were fatty acids and fatty acid conjugates. Interestingly, the amino acids citrulline and ornithine from the mitochondrial segment of the urea cycle both show to significantly decrease with metformin treatment. These findings were also mirrored by the increasing concentrations of metformin in the HepG2 cell culture data. Additionally, significant decreases in the xanthine derivatives, theophylline and caffeine were seen. As an alternative method of data analysis, the cohort data was run through IDEOM. This was performed in the 8 batches in which the samples were analyzed on the platform, thus avoiding batch effects. Peak intensities were logged and delta change was calculated. A weighted meta-analysis style breakdown of the data was then used to visualize the results. As the in-house method is very robust this was a good way of expanding the range of data. Good correlations could be made between the program and the bespoke analysis. Citrulline, ornithine, theophylline and caffeine decrease with metformin treatment in human plasma and in HepG2 treated cells.

POSTER 272

2-Aminobutyric acid reflects myocardial redox state in doxorubicin-induced cardiomyopathy

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Clinical usage of doxorubicin (DOX) as a chemotherapeutic drug is limited by cumulative dose-related cardiotoxicity caused by oxidative stress. Empiric dose limitation is insufficient to prevent DOX-induced cardiotoxicity due to a great variability in individual DOX tolerance. Recently, we revealed that 2-aminobutyric acid (2-AB) was produced as a byproduct of glutathione (GSH), which is one of the antioxidants. In this study, we assessed the utility of 2-AB as a biomarker for the detection of DOX-induced cardiac oxidative damage. Differentiated H9c2 rat cardiomyoblast cells were stimulated by hydrogen peroxide (H_2O_2) for 24 hours. The cells were rinsed with ice-cold phosphate buffered saline and quenched with cold methanol. After incubation for 30 min at 37°C, samples were centrifuged and the aqueous phase was collected and lyophilizated. Dried polar metabolites were derivatized and analyzed with gas chromatography-mass spectrometry (GC-MS) system. The concentration GSH was determined using a commercial assay kit. Male C57BL/6J (10 wks) were treated with one-time intraperitoneal injection of DOX (20 mg/kg), and echocardiography was performed at 5th day after injection, and sacrificed at 7th day after. Metabolites from plasma and heart were extracted and measured with GC-MS.

Reactive oxygen species (ROS) production was measured by lucigenin-enhanced chemiluminescence. First, we confirmed that DOX induced ROS production in hearts and deteriorated cardiac function estimated by echocardiography in vivo (% fractional shortening (%FS); 59.4 ± 2.1 % (baseline) vs. 55.0 ± 2.2 % (DOX), p=0.0078). On the other hand, GSH levels in hearts remained unchanged after a week of DOX injection. In contrast, 2-AB levels in both plasma and hearts significantly increased in DOX-injected mice compared with baseline (plasma; 2.66 ± 0.35, 1.4 folds, hearts; 0.287 ± 0.034, 1.56 folds, p=0.0179 and 0.0018, respectively). Since 2-AB is the byproduct of GSH, these findings indicate that 2AB reflect compensatory maintenance of GSH under oxidative stress condition. Intriguingly, plasma concentration of 2-AB was inversely associated with %FS (p=0.028, R=-0.687). Next, we investigated whether oxidative stress actually promote 2-AB production in cardiac myocytes. We confirmed that treatment with H2O2, one of oxidative stress inducers, increase 2-AB levels by 2.32 ± 0.25, 1.85 folds in H9C2 cells and 11.23 ± 0.80, 1.63 folds in cultured medium (p=<0.01, both). H2O2 increased GSH levels in H9C2 cells as well, suggesting that GSH production is promoted to eliminate oxidative stress. In summary, clinically available test methods for evaluation of oxidative stress have not been established so far. In the present study, GSH levels did not change in parallel with ROS production induced by DOX administration. On the other hand, 2-AB reflect GSH homeostasis under oxidative stress. The present findings suggest that monitoring of 2-AB could provide a novel strategy for early detection of DOX-induced cardiomyopathy. 2-Aminobutyric acid reflects glutathione homeostasis under oxidative stress in doxorubicin-induced cardiomyopathy.

POSTER 273

Mass Spectrometry-Based Metabolite Profiling in the Mouse Skin and Liver following Exposure to Ultraviolet B Radiation

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Ultraviolet (UV) light is mostly absorbed in the skin and causes direct damage to DNA as well as it indirectly leads to the production of free radicals, including reactive oxygen and nitrogen species. Although many physiological evidence-based molecular mechanism studies have been performed on the effects of UVB radiation on the skin, metabolite profiling of skin after UVB radiation has not yet been performed. Furthermore, only a limited number of reports have investigated these effects of UV radiation on non-skin tissue compared with those of on the skin. The hairless mice were divided into the two groups: normal group and UVB group. Mice were exposed to UVB radiation 3 times per week. After exposing mice to UVB radiation during weeks 6 and 12, ten mice of each group with 20 mice were sacrificed, and skin and liver tissue samples were collected, respectively. We investigated the metabolite changes in both the skin and liver of hairless mice following chronic exposure to UVB radiation using various mass spectrometry (MS)-based techniques including ultra-performance liquid chromatography (UPLC)-quadrupole time-of-flight (Q-TOF)-MS, gas chromatography (GC)-TOF-MS, and nanomate LTQ-MS analyses with multivariate statistical analysis, respectively. In the skin, many different kinds of skin metabolites were altered by the UVB exposure. Especially, cis-urocanic acid and cholesterol showed the most dramatic increase and decrease at 6 and 12 weeks, respectively, indicating their potential as candidate biomarkers related to the regulation of skin photodamage. In addition, the changes in skin primary metabolites and lysophospholipids induced by UVB exposure were generally greater at 12 weeks than at 6 weeks. In the liver, we detected several hepatic metabolite changes suggesting chronic UVB irradiation may impact significantly on major hepatic metabolism processes, despite the fact that the liver is not directly exposed to UVB radiation. Of them, glutamine exhibited the largest changes with a 5-fold increase at 6 weeks, indicating its potential as an indirect photodamage-related biomarker in the liver. It was the first attempt to investigate the effects of UVB radiation on the skin and liver, using metabolomics approach.

POSTER 274

Characterization of Metabolic Dynamics of Embryonic Stem Cells During Neural Differentiation

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Pluripotent cells, which can form all three germ layers and give rise to any cell in the body, have broad potential applications in regenerative medicine. However, despite extensive research, much remains unknown about regulation of stem cell differentiation toward desired cell types, and fine control remains elusive. Although characterization of differentiation has largely focused on gene and protein expression, metabolism has increasingly been shown to play an integral role and is gaining recognition as a novel way to monitor and affect differentiation. An untargeted metabolomic analysis over the time course of differentiation can provide a broader understanding of the underlying systems-level dynamics during this process. To this end, we measured metabolism daily over a ten-day neural differentiation of mouse embryonic stem cells. The metabolomic profiles of intracellular samples were measured using two-dimensional gas chromatography coupled to time of flight mass spectrometry (GCxGC-TOFMS). The progression of differentiation was verified via flow cytometry to assess loss of pluripotency and commitment to a neural lineage using established protein markers (Oct4 and Sox2, and Pax6 and beta-III tubulin, respectively). After seeing surprising metabolic oscillations, the metabolic impact and effect on differentiation of daily partial media changes versus complete media change every other day was assessed. Finally, we tested whether medium supplementation with metabolites we identified as having significantly changed during differentiation would affect differentiation efficiency. Dynamic changes in metabolism during differentiation were captured by our untargeted metabolomic profiling; principal component analysis showed clear separation between daily samples with an especially marked difference between early and late days of differentiation, and a strong temporal component evident in the first principal component. Daily partial feeding of the cells mostly eliminated the observed metabolic oscillations, allowing for easier interpretation of the metabolite trajectories. Closer examination of individual metabolite level trajectories revealed trends such as significantly decreased intracellular levels of most amino acids and an increase in levels of metabolites known to have roles as neurotransmitters, such as GABA and beta-alanine. Numerous metabolite trajectories exhibited a decrease in levels followed by an increase in levels (or vice versa), highlighting complex overall metabolic dynamics that arise from either transitory cell types or the continuously changing composition of the heterogeneous cell population during differentiation. For example, TCA cycle intermediates fumaric acid and malic acid increase in level through day 4 before dropping through day 10, while putrescine, a polyamine, does the opposite. Comparing these metabolite profiles with flow cytometry results allowed us to characterize how metabolic changes correspond with already recognized markers. Metabolite supplementation revealed insight into how this transition might be better controlled. We are currently looking to apply this method of analysis to other lineages, yielding more knowledge about the metabolic dynamics in specific differentiation lineages, and thus building on our holistic understanding of stem cell differentiation. This is the first-ever detailed temporal study of metabolism using untargeted metabolomics during stem cell differentiation.

POSTER 276

Metabolomic profiling of human naïve and primed embryonic stem cells reveals distinct differences in metabolism

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Mouse embryonic stem cells (mESCs) have been long known to exist as two stable pluripotent cell states known as naïve and primed mESC. Recently, the naïve state was also derived from human embryonic stem cells (hESCs). Naïve ESC are characterized by expression of Oct4 driven by a different enhancer than primed hESC, X inactivation (in females), increase in DNA methylation and deposit of H3K27me3

histone modifications(Gafni, O., 2013). Understanding these two ESC states has important implications for our understanding of human development, regenerative medicine and cancer. While investigations into other “omics” have been reported, the work presented here offers a novel exploration in the primary, secondary and lipid metabolism of these cells, showing dramatic differences between the two pluripotent states. Both naive and primed hESC were harvested and extracted for GC-TOFMS, HILIC-QTOFMS and RPLC-QTOFMS analysis. For GC-TOF, samples were submitted to derivatization and analyzed using Leco Pegasus IV, deconvoluted using ChromaTOF (Leco) and identifications performed by BinBase (Fiehn, O., 2008). HILIC-QTOFMS and RPLC-QTOFMS samples were analyzed using Agilent 6550 QTOFMS, ESI (+) mode for HILIC and both ESI(+) & ESI(-) for RPLC. Raw data was processed using MSDial for peak finding and identifications made using mz/RT in house library and Lipidbast (Kind,T., 2013). Statistical analysis was performed and submitted using R (R Development Core Team, 2011) to DeviumWeb (v 0.3.2)(Grapov, D., 2014) for creation of multivariate classification model (O-PLS-DA). Robust model performance statistics generated by Monte Carlo cross validation. Using the combination of GC, HILIC and RP (for lipids) we have performed an in-depth interrogation of the metabolism of naive and primed human embryonic stem cells (hESC). After data processing and annotations, the combination of these platforms yielded in excess of 400 annotated metabolites involved in a diverse range of cellular processes. These included primary metabolites including sugars and amino acids, secondary metabolites including methylated metabolites and lipids and fatty acid species. After performing statistics for metabolites significantly changed between the two states, we found multiple pathways which were differentially regulated in results from all three platforms. Specifically, the GC data showed differences in amino acid and reducing cofactor metabolism between the two cell types. HILIC data showed similar results, in addition to significant differences in metabolites tied to methylation potentials of the cells. Including changes in S-adenosyl methionine, S-adenosyl homocysteine and 1-methyl nicotinamide. The Lipid data showed differences in phosphatidylcholines, ceramides and fatty acid levels between cell states. Overall, dramatic and statistically significant differences in the metabolism of naive and primed hESC was observed as shown by O-PLS-DA for the combined results from all platforms and within each platform. These results provide advancement to the understanding of the metabolic states of these two cell types. Since naive hESC are more pluripotent and differentiate into primed hESC, this data could be used to create hypotheses regarding how these cells transition between their two distinct metabolic and pluripotent states. Extensive multiplatform metabolomic characterization of human primed and newly derived naïve embryonic stem cells.

POSTER 277

Global Metabolomic Profiling of Endothelial Cell Response to Inorganic Phosphate

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Recent studies suggest that elevated serum inorganic phosphate (Pi) is independently associated with increased vascular calcification (VC) and mortality. Pi induced osteogenic differentiation of vascular smooth muscle cells is considered to be a major mechanism underlying Pi-induced VC process. The role of endothelium in Pi-induced VC is not well studied, and the cellular response to extracellular Pi concentration is not well understood. We hypothesize that vascular endothelium dysfunction mediates Pi-induced vascular calcification. NMR broad spectrum metabolomics analysis has been employed to investigate endothelial cell response to Pi and to study the role of endothelium in Pi-induced vascular calcification. Pi-free culture environment was created by using Pi-free DMEM (Gibco), dialysed fetal calf serum (FBS; Gibco), and normal saline (0.9% NaCl. Phosphate buffer (pH 7.4) was used to adjust final Pi concentration in the medium. Mouse myocardial endothelial cells (MEC) were cultured up to 70% confluence in normal Pi (1.0mM) medium, then starved for 12h in 1.0mM Pi FBS-free medium, and subsequently cultured in study media containing 0.0mM, 0.1mM, 1.0mM and 5.0mM Pi, respectively for 4, 24, and 72h. Cells were pelleted, and cell extracts were prepared using acetonitrile: water (1:1). NMR broad spectrum metabolomics analysis was conducted on the reconstituted cell extracts, and microarray

and proteomics analyses were performed in parallel. After culturing MECs in Pi free media, cells were shown to become trypsin-resistant and growth arrested similar to our observations in rat vascular smooth muscle cells (VSMC) grown in the same conditions. Western blot analysis found that sodium dependent phosphate transporters were expressed in MECs. Transient activations of signaling molecules, ERK1/2 and AKT, phosphorylation, were also observed. Preliminary microarray profiling of transcriptomes in MEC in response to normal (1mM) and high (5mM) Pi in comparison with Pi-free condition revealed activation of genes involved in cell cycling, DNA synthesis, many signaling pathways, metabolic networks of cholesterol, glycogen, and amino acids. Significance Analysis of Microarrays (SAM) and GeneSpring analyses identified differentially regulated genes and pathways, such as globally upregulated genes involved in proteasome degradation complex. Furthermore, proteomics data revealed that MEC in normal Pi condition, express known mineralization inhibitory proteins, such as OPG, SPP1, MGP, VNN1 and VKORC1, and signaling molecules involved in bone and vascular mineralization, such as BMP, FGFR and Wnt signaling. Multivariate analysis of NMR binned data was used to determine the bins that were important for distinguishing study phenotypes and the bins were then library-matched to metabolites. Each phenotypic group of cells grown in Pi-free, 0.1mM, 1mM, and 5 mM Pi (for 4h, 24h, and 72h) separated in the principal component analysis indicating that they have distinct metabotypes. Furthermore, when compared to 1mM Pi (physiological Pi), 0mM, 0.1mM, and 5mM Pi treated cells had common marker metabolites shared among the phenotypes as well as unique metabolites for each study phenotype; these observations were time- dependent. Energy-associated (AXP), amino acid (eg. alanine, Isoleucine, glutamate, glutamine, glycine, leucine, proline, threonine, tyrosine, valine), choline and phosphocholine, lipid, and nucleotide (IMP, UMP, UDPOSTER glucose, uridine), metabolic pathways were among the most perturbed. Identifying metabolites regulated by extracellular Pi provides novel information regarding cellular and molecular responses to changes of extracellular Pi concentrations.

POSTER 278

Metabolomic study of burn injury-induced SIRS/ARDS in a rat model

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Burn injury is considered as a global health concern resulting in significant rates of morbidity and mortality annually. Death from burn injury can often be traced to secondary complications arising from an overwhelming inflammatory response that leads to dysfunction of multiple organs, particularly the lungs. Identification of biomarkers that will enable the early detection of systemic inflammation and the onset of organ dysfunction can aid in the determination of suitable treatments and improve the outcome of burn victims. NMR and LC-MS were used to obtain a comprehensive profile of the metabolic changes in an established rat model of burn injury-induced systemic inflammatory response syndrome and acute respiratory distress syndrome (SIRS/ARDS). Adult male Sprague-Dawley rats were subjected to thermal injury by immersing them in 100˚C water bath to produce 45% total body surface area (TBSA) full thickness burn. Rats were sacrificed at different time points (6, 24, 48 hours) post-injury. Plasma samples, as well as tissue samples from the lungs, kidney and liver, were obtained and subjected to extraction. NMR analysis was performed using a 600MHz NMR. LC-MS analysis was done using HILIC chromatography coupled to a QTOF MS. Data from both NMR and LC-MS analyses were extracted and subjected to multivariate analysis in order to identify significantly differentiating metabolites. Correlation analysis with cytokine and organ function data will also be performed. NMR analysis of the plasma samples has revealed the clear differentiation of the thermally injured rats from the sham rats. A different trend was observed for the LC-MS analysis wherein the score plots showed the samples at 6h post-burn being largely separated from the sham, 24h and 48h samples, which were more clustered together. This trend indicates a different set of metabolites that significantly change after burn injury but returns back to normal levels through time with minimal intervention. Distinct differentiation of the burn injury samples from the sham samples were also observed for the different organs. Metabolites found to be significantly differentiating across the different organs and the plasma samples include purine metabolites, amino acids and derivatives, fatty acids, glycerophospholipids, and glucose. Preliminary analysis of the inflammatory response and organ function markers had also revealed significant increase in some

cytokines with indications of dysfunction in the liver, kidney, and lungs. These results will then be correlated with the metabolic changes to identify potential metabolic markers of SIRS/ARDS and other organ damage markers. A cross-platform and multi-organ metabolomic characterization of burn injury-induced SIRS/ARDS, which to our knowledge, has not been previously reported.

POSTER 279

Lipidomic profiling in skin from db/db and normal mice using direct-infusion nanoelectrospray-tandem mass spectrometry

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Lipidomics is an emergent “omics” science and is the large-scale study of pathways and networks which is based on analysis of all lipids present in a biological system. The use of shotgun lipidomics, based on multi-dimensional mass spectrometric array analyses after multiplexed sample preparation and intra-source separation, has been recently advanced to a mature technique for the rapid and reproducible global analysis of cellular lipids. Among these lipids, ceramides are sphingolipids consisting of sphingoid bases, which are linked to fatty acids by an amide bond. In this study, we profiled and identified skin ceramides in skin stratum corneum (SC) of diabetic mice using direct infusion nanoelectrospray-tandem mass spectrometry. Skin lipids were extracted using the Masson method with some modifications. Before the lipid extraction the skin samples were lyophilized, homogenized in methanol. Then samples were centrifuged, the lower phase were homogenized in dichloromethane/methanol (3:1). Lipid extracts were concentrated and reconstituted with methanol/chloroform (9/1) containing 7.5mM ammonium acetate. Aliquots were subjected to the direct infusion nanoelectrospray tandem mass spectrometry to identify skin ceramides in the samples. Nominal ion mass spectra data from the ion trap mass spectrometer were processed by the Genedata Expressionist MSX module. In addition, serum lipids were identified using the LIPID MAPS Lipidomics Gateway and our in-house lipid library. Multivariate analysis was performed to explore the extent of differences among the three groups. Typical sphingoid bases in the skin are composed of dihydrosphingosine (dS), sphingosine (S), phytosphingosine (P), and 6-hydroxysphingosine (H), and the fatty acid acyl chains are composed of non-hydroxy fatty acid (N), α-hydroxy fatty acid (A), ω-hydroxy fatty acid (O), and esterified ω-hydroxy fatty acid (EO). The 16 ceramide classes (NS, NdS, NH, NP, AS, AdS, AH, AP, EOS, EOdS, EOH, EOP, OS, OdS, OH, and OP) derived from the combination of sphingoid bases and fatty acid acyl chains. We profiled skin ceramides in skin SC of diabetic mice using direct infusion nanoelectrospray-tandem mass spectrometry. Total 61 skin ceramides including NS, NdS, NH, NP, AS, and AdS were identified in the stratum corneum of mice skin. Diabetic and normal mice groups were clearly discriminated from each other on PCA, PLS-DA, and OPLS-DA score plot, and major metabolites contributing to the discrimination were assigned as ceramides such as NS, NdS, and NP-type. The levels of NS-type ceramides were increased in diabetic mice skin compared to normal mice skin, whereas those of NP- and NdS-type ceramide were decreased in diabetic mice skin. These results might be originated from the down regulation of dihydroceramide desaturase 1 which is a key enzyme in mediating fatty-acid induced insulin resistance or up regulation of ceramidase in skin. In addition, skin pH showed negative and positive correlation with NP-type and NS-type ceramide, respectively. Ceramide distribution in skin SC of diabetic mice might be a metabo-marker closely related with the phenotype of diabetic mice.

POSTER 280

Impact of Milk Peptides on Sebocyte Lipid Mediators

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Sebocytes are lipid-producing cells that compose the sebaceous glands. Increased secretion of lipids from these cells is associated with acne. Lipids mediators (e.g. oxylipins, endocannabinoids, ceramides/sphingoid bases) may participate in the inflammation of acne. However the capacity of sebocytes to produce lipid mediators in response to inflammatory stimuli is not well reported. The relationship between milk and acne has been controversial. Milk upregulates insulin like growth factor 1 (IGF-1), a precursor to lipogenesis and several reports suggest that bovine milk derived proteins such as whey and casein are associated with acne flares. However, certain components in milk, such as lactoferrin, may reduce inflammation. Therefore prospective and mechanistic research on how milk subcomponents interact with sebocytes is needed. Sebocytes from the SEB-1 p29 cell line were treated in duplicate with combined milk peptides, and individually with α-casein, β-casein and bovine lactoferrin, each at a concentration of 10 pM. Milk peptides were isolated from concentrated whey permeate by flash chromatography and quantified by the bicinchoninic acid assay. Lipid mediators including 95 oxylipins, 30 endocannabinoids and 16 ceramides/sphingoid bases, were isolated from the sebocyte media by solid phase extraction on 60 mg Waters Oasis-HLB cartridges and quantified by UPLC-(ESI) MS/MS on an AB SciEx API 4000 QTrap operated in multi-reaction monitoring mode. Analytes were quantified against authentic standards using and deuterium labeled surrogates. A total of 11 oxylipins (prostaglandins and HETEs), 16 endocannabinoids (acylglycerides and ethanolamides), and 9 ceramides/sphingoid-bases were detected in the media of sebocytes treated with both the combined and individual milk peptides. No differences were observed in the detected oxylipin and ceramides/sphingoid base profiles between the treatment groups and control sebocytes. With respect to the endocannabinoids, monoacylglycerides were unchanged, while milk peptides increased levels of the suite of detected acylethanolamides, including a 2.1-fold increase in oleoylethanolamide (OEA), a 2.3-fold increase in linolenoylethanolamide (LEA), a 1.7-fold increase in arachidonoylethanolamide (anandamide, AEA), a 1.5-fold increase in docosatetraenylethanolamide (DEA), and a 1.6-fold increase in docosahexaenoylethanolamide (DHEA). Bovine lactoferrin generally increased media acylethanolamide levels, while casein appeared to suppress them relative to controls. Bovine lactoferrin also increased palmitoylethanolamide (PEA) levels 2-fold independent of milk peptides. The anti-inflammatory effects of PEA and AEA have previously been demonstrated in the context of acute inflammation, and it has been suggested that OEA, LEA, DEA and DHEA may exert similar anti-inflammatory effects. However, acylethanolamides have also been shown to increase sebocyte lipid synthesis and apoptosis at high doses. Therefore it is not clear how this may effect acne and this effect would need further direct assessment in further cell based and clinical studies understand its influence on sebocytes and acne. Our findings show that exposure to the bovine lactoferrin component of milk peptides can stimulate anti-inflammatory endocannabinoid production by sebocytes, while leaving pro-inflammatory eicosanoid production unchanged. Therefore, despite the controversial relationship between milk and acne, these results indicate that there may also be beneficial anti-inflammatory effects of milk peptides, mostly moderated through lactoferrin. These results provide a rational underpinning for future in vivo experiments to assess the utility of lactoferrin as a therapeutic intervention for acne with possible expansion into other inflammatory skin disorders such as atopic dermatitis. Sebocyte endocannabinoids but not eicosanoids production is modulated by milk peptides.

POSTER 281

Characterization of the secondary metabolome of Penicillium using isotopic labeling and HPLC-HRMS

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Characterization of fungal secondary metabolomes has become a great challenge in the last decades due to both the emergence of fungal threats, and the industrial interest of many natural products. In view of this, we recently developed an analytical strategy for fungal secondary metabolome characterization (Cano P. et al. Anal. Chem. (2013) 85:8412). This strategy has been applied on the characterization of secondary metabolomes of Penicillium verrucosum and Penicillium nordicum, fungal contaminants of stored cereals. Penicillium verrucosum (NRRL 5571) and Penicillium nordicum (NRRL 6062) were grown on wheat grains (Triticum aestivum) with different isotopic enrichments: (i) naturally enriched grains, (ii) 97% 13C, and (iii) 53% 13C / 97% 15N. Extract of each culture was then analyzed by HPLC coupled to a LTQ-Orbitrap mass spectrometer equipped with electrospray ionization, operating in the positive or the negative mode. Metabolites were then specifically detected according to the specific isotopic pattern of the different isotopic enrichments. Known secondary metabolites were suspected using the Antibase database, then annotated by MS/MS experiments, and identified by comparison with standard compounds when available. Unknown secondary metabolites were tentatively annotated using molecular networks of MS/MS similarities (Watrous J. et al.; PNAS (2012) 109 E1743). Wheat grains represented the only source of carbon and nitrogen for fungal growth. Therefore, produced fungal secondary metabolites were either unlabeled (naturally enriched cultures), singly labeled (13C cultures) or doubly labeled (13C/15N cultures). This feature allowed discrimination of fungal metabolites against non-fungal compounds which remained unlabelled in the three substrates. Fungal origin was further confirmed by analysis of a blank 12C wheat extract (without fungus). The use of a 50% 13C enrichment for the 13C/15N wheat substrate resulted in a characteristic isotopic pattern which enabled the specific detection of fungal metabolites. Furthermore, the comparison of m/z ratios of a same metabolite detected in the different culture conditions as non-labeled, fully 13C labeled and doubly 13C/15N labeled, led to the unambiguous determination of the number of carbon and nitrogen atoms. This was facilitated by our in-house developed software “Mass Compare”, which determines the only possible chemical formula for each metabolite. This approach was successfully applied to the detection of more than 300 secondary metabolites. Interestingly, only 20% of them are suspected to be known metabolites according to AntiBase, meaning that 80% of this metabolome is unknown. At this stage, some known metabolites have been confirmed by analyses of available standard compounds (Ochratoxin A, Citrinin …). To enhance unknown identification efficiency, the molecular network of MS/MS similarities has been modeled to highlight some clusters of known/unknown metabolites, allowing interpretation of their MS fragmentation patterns to propose their structure. Specific detection and identification of new fungal secondary metabolites produced by Penicillium.

POSTER 282

Composition of metabolites produced by a coastal microbial community in the presence of oil

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The marine environment holds one of the largest pools of reduced organic carbon on Earth. Within this organic carbon are metabolites that are produced as a result of microbial activity. In this project, we examined the response of coastal microorganisms to the presence of oil. We incubated a coastal microbial community with the water-soluble fraction of crude oil and allowed the incubation to proceed for fourteen days. During that time we sampled the intracellular and extracellular metabolites. The extracts were analyzed using liquid chromatography-based targeted and untargeted metabolomics methods that we have modified for use with marine samples. With thousands of metabolites in the untargeted mass spectrometry dataset, we need a means to narrow our focus on putatively annotating those metabolites that are ecologically interesting. To accomplish this goal, we used Weighted Correlation Network Analysis. This tool allows us to find correlations between our untargeted metabolomics data and the pre-defined list of metabolites from the targeted data analysis. The results identified novel metabolites produced by the coastal microbial community in the presence of oil as well as metabolites produced under all experimental conditions. This combination of metabolomics data and associated metadata will provide new insights into novel metabolites found in marine environments.

POSTER 283

Integrating Exometabolomics and Metabolic Modeling of Microbial Interactions Towards Consolidated Bioprocessing for Next Generation Biofuel Production

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The biologically mediated production of energy-dense fuels from the degradation of lignocellulose is a complex, multistep metabolic process. Metabolite exchange is central to microbial community conversion of plant biomass but is poorly understood and cannot yet be accurately modeled. Harnessing microbial communities for biofuel production has the potential to enable efficient, robust, and adaptive approaches to consolidated bioprocessing. To accomplish this, a quantitative and predictive strategy is needed to assemble stable synthetic consortia. To this end, we are combining multiple approaches, including computer modeling of ecosystem-level microbial metabolism, mass spectrometry-based exometabolomics, and experimental evolution. We aim to implement a high-throughput pipeline for the systematic, computationally-driven study and optimization of microbial interactions and their effect on lignocellulose degradation and biofuel production. We are assembling a synthetic microbial consortium consisting of a lignin degrader, a (hemi)-cellulose degrader, a strain to detoxify methoxy groups on lignin, and a biofuel producing yeast. We are employing LC-MS based exometabolomics to characterize in detail the metabolic inputs and outputs of different candidate consortium members. These data are being used to test and improve genome scale metabolic network models of individual strains. In parallel, we are refining stoichiometric models for the relevant organisms, starting from published reconstructions, and from gaPoster filled draft models obtained through the KBase pipeline. Simulations of the combined metabolic activities of these organisms will be performed through COMETS (Computation Of Microbial Ecosystems in Time and Space), our platform for dynamic flux balance modeling. In the process of narrowing down the list of candidate strains for our synthetic consortium, we have selected the oleaginous yeast Yarrowia lipolytica as our biofuel-producing member. A genome-scale stoichiometric model for flux balance analysis has been developed for this organism, which we are refining with experimental data. We conducted experiments with Y.lipolytica in minimal medium, with individual or combinations of carbon sources representing those in plant hydrolysates. Quantitative LC-MS analysis allowed comparison of experimental uptake rates for these carbon sources to those predicted by the model. Untargeted LC-MS analysis of spent media also allowed characterization of metabolites released by Y.lipolytica when cultured on different carbon sources. These data allow insights into metabolic pathways and substrate preference of the organism. Methylobacterium extorquens has been selected as our lignin detoxifying strain. A genome-scale stoichiometric model for flux balance analysis is available, and model refinement and improvement is ongoing. Other candidate consortium members are being evaluated for their lignin degradation (Streptomyces, Pseudomonas, and Rhodococcus species) and (hemi)-cellulose degradation (Streptomyces and Cellulomonas species) capabilities. We are culturing strains on lignin and hemicellulose substrates and characterizing the resulting bio-degradation products by LC-MS. Once all consortium members are chosen, metabolic network models of individual species will be tested and refined. By extending these analyses to all pairs and larger combinations of organisms, we will comprehensively detect possible synergies and partner-induced changes in metabolite uptake/excretion. Where necessary consortium members will be improved for their particular metabolic tasks through experimental laboratory evolution. In this way we aim to construct a synthetic microbial consortium capable of consolidated bioprocessing of lignocellulose for the production of biofuel. With our integrative approach we aim to harness the biosynthetic processing power of microbial communities in a predictive, quantitative way.

POSTER 284

Experimental examination of catabolite repression and diauxic growth in C. phytofermentans

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Metabolite measurements of C. phytofermentans (Cphy) batch experiments grown in the presence of select hexose or pentose sugars were used to constrain a genome-scale computer model to conduct a system-level analysis of metabolism. Using the result of our analyses, we identified a set of target genes that should be inactivated in order to generate sugar substrate-specific mutants. We deleted specific genes to produce sugar specific strains of Cphy. The phenotype of sugar specific strains was verified during growth experiments by using metabolomic measurements and comparing the growth curves. The deletion strains showed impairment when grown on their respective sugars. Data from batch experiments with Cphy under different environmental and nutritional conditions were used in conjunction with genome-scale modeling to conduct a system-level analysis of Cphy metabolism. We used CRISPR/cas9 method to target homologous recombination events interrupt key genes to produce sugar specific strains of Cphy. In addition to cell count using traditional optical density (OD) with spectrophotometer we also used microscopic evaluation. Microscopy with live/dead staining helped to assess the numbers of active cells in culture, cell accumulation, spindle communities and sporulation cycle during all phases of growth. High Performance Liquid Chromatography (HPLC) assessed the sugar uptake and fermentation products formed. Colorimetric assays confirmed the uptake of sugars in mixtures that cannot be otherwise resolved with the HPLC. We will present measurements of the Cphy growth rates and nutritional exchange rates of Cphy under a variety of different nutritional conditions, including growth in presence of select hexose or pentose sugars. For each analysis, we collected culture aliquots at regular time intervals to measure cellular growth rates and use HPLC-MS to identify and measure the concentration of various metabolites and fermentation products (e.g. select sugars, small organic acids, ethanol, acetate, butanol etc.) in the medium. By incorporating the experimental results, we constrained a Flux Balance Analysis (FBA) model and greatly improved the accuracy of its predictions. Our microscopic evaluation with live/dead staining helped assess the role of the active population within the total cell count (OD) showing that production of solvents occurred during formation of spores and therefore might be linked to sporulation cycle. In order to further optimize biofuel production we examined how the life cycle of Cphy is influenced by changing reactor conditions such as drop in pH (from 7 to 6), solvent build up, cell spindle / clump formation (1-10cm) and temperature change. Biofilm aggregates (10-150 microns) form around insoluble cellulose particles have been shown to increase ethanol production. The formation of aggregates might be influenced by cell surface charge and motility/chemotaxis phenotypes. We examined Zeta potentials of Cphy grown on different substrates and aggregate formation in the presence or absence of Flagellum. Our studies into formation of spindles and clouds of bacteria during late stationary phase have provided new insights into causes of this phenomenon. We believe this process can lead to increased solvent production and therefor it may be useful to better optimize spindle formation conditions. We used CRISPR/cas9 targeted homologous recombination to produce sugar specific strains of biofuel producing Clostridium phytofermentans identified by FBA modeling.

POSTER 285

13C Metabolic Flux Analysis for systematic metabolic engineering of yeast to produce fatty acid-derived fuels and chemicals

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Fatty acids are extensively used to produce transportation fuels and chemicals including surfactants, solvents and lubricants. In recent times, bioengineered microorganisms have been developed at the Joint

BioEnergy Institute and other institutions to produce these compounds from renewable biomass. However, microbial metabolism is non-trivial to redirect into abundant production of the desired chemicals. Recent developments in metabolomics have enabled us to evaluate the metabolic state of the producing microorganism based on 13C labeling experiments, which are able to discern the flux through the different metabolic pathways in its organism. We use the knowledge to shed light into microbial metabolism and improve metabolic engineering efforts for fatty acid-derived biofuel production. A biosynthetic pathway for free fatty acid production was constructed by overexpression of acetyl-CoA carboxylase, fatty acid synthase and elimination of FAA1 and FAA4 involved in the fatty acid degradation pathway. We performed 13C tracer experiments and used a new method to determine fluxes for a genome-scale model of yeast metabolism: two scale 13C Metabolic Flux Analysis (2S-13C MFA). Acetyl-CoA is involved in many different metabolic pathways and it is a precursor metabolite for biosynthesis of fatty acids. Rational engineering of yeast metabolism was performed based on flux distributions obtained from a new method to determine fluxes for genome-scale models of yeast metabolism for fatty acid overproducing strains. Genome wide acetyl-CoA balance helped us to identify potential gene targets such as malate synthase for overexpression, down-regulation, deletion or temporal/spatial adjustment. Further increase of free fatty acid production was obtained by introduction of heterologous ATPOSTER dependent citrate lyase (ACL) as an alternate acetyl-CoA biosynthetic pathway. Genome-scale internal metabolic flux maps were obtained for over production of fatty acids in yeast based on 13C labeling experiments.

POSTER 286

An SPE-LC/MS-based Workflow for Accurate Quantification of Escherichia Coli Cofactors Facilitates Metabolic Engineering

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Metabolic engineering of microbial strains for increased productivity of target compounds often involves fine-tuning of the energy and redox balance inside cells. Conventional enzymatic assays for cofactor measurement usually provide quantitative results of poor accuracy and reproducibility. To facilitate cofactor engineering, we developed an SPE-LC/MS-based workflow to analyze essential cofactors engaged in central metabolism in E. coli. This workflow was implemented to accurately quantify cofactors in a wild-type strain and five engineered strains with varying yields of isobutanol, a promising biofuel. Our cofactor quantification reveals the critical contribution of NADPH supply to isobutanol production. Our study also suggests that it is the absolute amount of cofactor rather than the cofactor ratio that determines the biosynthetic activity of engineered strains. Escherichia coli strains (ATCC 8739, As108, As145, As166, As255, As266) were cultured in minimal complete media and harvested at OD650 of ~1.0. After quenching and centrifugation, intracellular metabolites were extracted with a solvent mixture of MeOH:ACN:H2O (4:4:2, v/v) and 0.05 M NH4OH. Freeze-thawing and sonication were then applied to warrant efficient metabolite extraction. After protein precipitation, the supernatant from each sample was subjected to SPE enrichment of targeted cofactors. SPE elution was concentrated before injected into HPLC-MS for cofactor analysis. HILIC-based separation and high-resolution Q-IM-TOF mass spectrometry (Synapt, Waters) were employed in this study. Quantification of each cofactor concentration in the biological matrix was achieved by the standard addition strategy. 1. A specific SPE-LC/MS approach was established for identification and quantification of 6 cofactors (ATP, ADP, NAD, NADH, NADP, NADPH) in bacterial cells. Standard curve experiments demonstrated linearity within 0.01−1600 μM (R2>0.99). LOD, LOQ are in the range of 0.01-3.13 μM and 0.02-6.25 μM, respectively for different cofactors. 2. The established method showed sufficient quantitative precision for all cofactors (RSD <20%) among biological replicates of E.coli cell culture. We further compared the intra- and inter-RSD of NAD and NADH measurement using our approach and conventional enzymatic assays from two vendors. Our approach outperformed both commercial enzyme kits by showing the highest reproducibility of cofactor quantification. 3. Our approach was then employed to quantify cofactors in a wild-type strain and

five engineered E.coli strains with different isobutanol production in aerobic growth. The NADPH concentrations were found to be highly correlated with isobutanol yields in different engineered strains, whereas changes of other cofactors also indicated that improved isobutanol biosynthesis was resulted from manipulation of specific metabolic genes. Surprisingly, our study suggests that it is the absolute amount of cofactor rather than the widely-recognized cofactor ratio (i.e. NADP+/NADPH) that determines the biosynthetic activity of engineered strains. This finding has provided new insight in designing strains of increased isobutanol productivity. develop a new workflow for absolute cofactor quantification which is particularly useful for metabolic engineering.

POSTER 287

Advancing production of sustainable chemicals in metabolically engineered E. coli: application of LAESI-MS for direct screening of small-scale fermentation samples.

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Genomatica is advancing sustainable production of industrial chemicals using microbial biocatalysts (cell factories). We implemented a systems-based platform to metabolically engineer strains like E.coli and develop cost-advantaged commercial processes for 1,4-butanediol and other intermediate chemicals from renewable carbohydrate feedstocks. The ability to quickly screen targeted metabolites in fermentation samples and intracellular extracts can be markedly enhanced by implementing direct MS-based analytical techniques to complement traditional LCMS approaches and accommodate high throughput demands. Here we evaluated Laser Ablation Electrospray Ionization mass spectrometry (LAESI-MS), to semi-quantitatively analyze small scale fermentation samples without any front-end separation or preparation. Direct probing of E. coli cell pellets by LAESI-MS to assess intercellular levels of pathway intermediates and cofactors has also been initiated. Small-scale fermentation samples from E.coli strains, metabolically engineered to produce 1,3-butanediol (1,3-BDO), were quantified by conventional LCMS/MS methods. Cells were grown in 96-well plates on glucose as carbon source in specially designed minimal media. A LAESI DPOSTER 1000 system was interfaced with QExactive mass spectrometer. 20 uL aliquots of fermentation samples were placed in a 96-well microtiter plate for direct analysis by LAESI-MS. Sample ablation was achieved by 5 pulses of mid-IR laser at 1Hz, ionizing in the electrospray solvent (50% methanol/0.1% acetic acid). Targeted small molecule metabolites, major fermentation byproducts, were monitored in full MS profile and targeted MS/MS modes, with and without 13C internal standard. Specifically of interest were 1,3-BDO, 3-hydroxybutyrate, as well a few other metabolic intermediates. E. coli cells were metabolically engineered to produce 1,3-BDO from renewable carbohydrate feedstocks. High throughput fermentation protocols were developed in 96-well plate format to mimic conventional lab-scale fermentation in bioreactors. This workflow enabled distinguishing small differences in strain performance, therefore, substantially accelerating strain and process development. Small-scale fermentation samples were quantified by conventional LCMS/MS methods and LAESI-MS. Preliminary results demonstrated potential of LAESI-MS to semi-quantitatively screen fermentation samples in HTP manner directly in complex fermentation matrix without any chromatographic separation or sample preparation. The LAESI-MS process enabled detection of very low masses down to 50m/z with sufficient sensitivity in low-mM range. Acceptable linearity and correlation in analyte concentrations between LAESI-MS and LCMS/MS was achieved. It was found that internal standard and targeted MS/MS mode resulted in more accurate measurements. LAESI-MS analysis time was 35s per sample due to 1,3-BDO signal tailing effect, also observed in LCMS analysis. Further optimization of LAESI and MS parameters is needed to reduce cycle time and increase analysis throughput. In addition, LAESI-MS technology can potentially enable measurements of intracellular metabolites of interest directly from a bacterial cell pellet without any extraction. We have started experiments to evaluate it. If successful this approach can substantially accelerate metabolomics analysis and alleviate complications associated with intracellular extraction processes and unwanted changes in metabolome composition during sampling and extraction. In conclusion, preliminary experiments demonstrated that LAESI can be a robust tool for rapid semi-quantitative targeted screening of fermentation samples and potentially intact bacterial cells.

First application of LAESI-MS to semi-quantitatively screen fermentation samples for 1,3-butanediol production by metabolically engineered E. coli cells.

POSTER 288

Quantitative and Qualitative Metabolomics for the Investigation of Intracellular Metabolism

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Studying intracellular metabolism of model organisms, such as E. coli, is vital to further our biochemical knowledge1, to develop new pharmaceuticals that target harmful pathogens2 and to improve industrial applications that aim to metabolically engineer bacteria in order to produce commodity chemicals from renewable resources3. Paramount to these endeavors is the ability to reliably and accurately measure the intracellular metabolome. By measuring the absolute metabolite levels of such compounds, one is able to calculate reaction and pathway thermodynamics4 and infer in vivo enzyme kinetics5. When microorganisms are grown on a specifically chosen labeled substrate (e.g. 1-13C glucose) during a metabolic labeling experiment, the isotopomer distribution of intracellular compounds can be used to calculate absolute flux through specific reactions of interest6 Samples of E. coli were grown in 4 g/L glucose or complex M9 minimal media8 with trace elements9 and sampled from an anaerobic chamber4. Samples were extracted using a fast filtration approach and serially extracted using a mix of acetonitrile, formic acid, methanol and water. An LC-QTRAP® 5500 System (SCIEX) was used for targeted profiling. Samples were acquired using the Scheduled MRM™ Pro Algorithm in Analyst® Software 1.6.2. The information dependent acquisition (IDA) method consisted of a multiple reaction monitoring (MRM) survey scan coupled with an enhanced product ion (EPI) scan for compound identity confirmation. Samples were quantified using IDMS7, 10 with metabolically labeled internal standards from E. coli and processed using MultiQuant® Software 2.1.1. The quantitative workflow uses the Scheduled MRM™ Pro Algorithm to maximize method efficiency. When a signal is detected in the MRM scan, acquisition of an enhanced product ion (EPI) spectrum is triggered via IDA. The acquired MS/MS spectra can then be searched against a library of compound spectra from pure standards to provide greater confidence for compound detection. The compounds targeted in this workflow are involved in pathways that include central carbohydrate metabolism (i.e., glycolysis, the pentose phosphate pathway, and the citric acid cycle), amino acid metabolism and nucleotide and cofactor metabolism. An efficient and cost-effective means to generate internal standards for quantitation is through metabolic labeling10. This involves growing a micro-organism (e.g. E. coli) on a uniformly labeled carbon source resulting in a fully labeled biomass. This can be harvested, extracted and subsequently used as internal standards. In addition to coupling MRM and EPI scans, the qualitative workflow can also include an enhanced resolution (ER) scan which provides a higher resolution MS spectrum of each analyte with accurate isotope ratios. From the ER scan, the isotope distribution of metabolically labeled compounds can be determined in the labeling experiments. The EPI scan triggered provides additional structural information on the location of the heavy labels. These methods were applied to measure the differences in absolute metabolite concentrations in E. coli grown in two different media conditions. The first media condition was a glucose minimal media and the second was a complex media that included glucose and supplemented amino acids and precursors. We found that supplementation with amino acids and their precursors correspondingly increased levels of intracellular amino acids. This finding is consistent with previous studies that found an increase in growth rate for bacteria supplemented with amino acids due to the import of amino acids and their precursors into the cell13. The use of the QTRAP® 5500 System for quantitative and qualitative metabolomics investigations to understand intracellular metabolism of microorganisms.

POSTER 289

Overcoming the challenge of analyzing Intermediates of Isoprenoid Biosynthesis in Escherichia coli

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Isoprenoids are a large and highly diverse group of natural products with many applications including high value chemicals and advanced biofuels. They are synthesized from two universal building blocks, isopentenol pyrophosphate (IPP) and dimethylallyl pyrophosphate (DMAPP) via the mevalonate (Mev) pathway, which is generally prevalent in eukaryotes and archea, or the deoxyxylulose-5-phosphate (DXP) pathway, which is more widespread in bacteria. One of the main challenges of a rational approach to engineering the heterologously expressed mevalonate pathway or the DXP pathway in E. coli is the difficulty of measuring their intermediates, as they are comprised of organic acid, nucleotide, triose phosphate, pyrophosphate and coenzyme A compounds. Here we present the development of a novel CE-QTOF MS method to analyze these intermediates. Pressure-assisted capillary electrophoresis (Agilent 7100 CE System) was coupled to quadrupole time-of-flight mass spectrometry (Agilent 6550 TOF MS) via electrospray ionization. The conventional mode of CE was employed for analyte separation and mass spectrometric detection was conducted in the negative ion mode. IPP and DMAPP, which are notoriously difficult to separate, were resolved by adding cyclodextrins to the electrolyte. Preliminary results show good resolution for all target analytes, with intraday retention time and peak area repeatability measurements of <3 and <15% RSD, respectively, and sensitivity enhancements of up to 10 %. The method was also applied to the analysis of anionic intermediates of central carbon metabolism, showing that it can be used to understand the effect of pathway engineering on E. coli's physiological state. Thus, this method shows great potential for metabolomics scale experiments in engineered E. coli strains. This method can be used to correlate isoprenoid pathway engineering with central carbon metabolism and, hence, the cell's physiological state.

POSTER 291

Can we predict the intracellular metabolic state of a cell based on extracellular metabolite data?

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The analysis of extracellular metabolites presents many technical advantages over the analysis of intracellular compounds, which made this approach very popular in recent years as a high-throughput tool to assess the metabolic state of microbial cells. However, very little effort has been made to determine the actual relationship between intracellular and extracellular metabolite levels. The secretion of intracellular metabolites has been traditionally interpreted as a consequence of an intracellular metabolic overflow, which is based on the premise that for a metabolite to be secreted, it must be over-produced inside the cell. Therefore, we expect to find a secreted metabolite at increased levels inside the cells. Here we present a time-series metabolomics study of Saccharomyces cerevisiae growing on a glucose-limited chemostat. S. cerevisiae CEN.PK113-7D strain was grown on a glucose-limited chemostat (5 g/L) under anaerobic condition. Intra- and extracellular metabolomic samples (triplicates) were taken at anaerobic steady-state. Soon after, oxygen was introduced to the system to reach 70% saturation, and individual samples were taken during the transition between anaerobic and aerobic steady-states. Finally, samples (triplicates) were harvested after three residence times at aerobic steady-state. Samples for intracellular metabolite analysis were quenched using cold glycerol saline solution at -23oC and both intra and extracellular metabolite samples were analyzed by GC-MS after methyl chloroformate derivatization. Metabolites were identified based on their chromatographic retention times and mass spectra using an in-house MS library and software written in R platform. Overall, we accurately identified 64 intracellular and 38 extracellular metabolites in our samples. Although this is still a small snapshot of the whole yeast metabolome, it is enough to establish some typical relationship between intra and extracellular metabolite levels. Not all metabolites detected intracellularly were secreted to the medium, which was expected. Despite most metabolites identified in the extracellular samples had also been detected intracellularly, six metabolites were uniquely detected in the spent culture media. Therefore, the intracellular levels of these compounds were probably very low to be detected, which somehow question the concept of metabolic overflow. Among all identified metabolites, 68 had their levels changed significantly between anaerobic and aerobic steady-states either intracellularly,

extracellularly or both. It is clear that many metabolites had their intracellular and extracellular levels changing in very similar pattern when comparing the time series events (anaerobic steady-state/transition/aerobic steady-state); and many others showed similar trend when comparing the two steady-states alone. However, aspartate, 2-oxoglutarate, benzoate and the fatty acid decanoate had their intracellular and extracellular levels changed in opposite directions, which does not conform to the metabolic overflow theory. The intracellular level of these four metabolites decreased significantly between anaerobic and aerobic growth, whilst their extracellular level significantly increased. Therefore, the yeast cells seem to have actively secreted these compounds in response to oxygen, which was independent of an intracellular overflow. The intracellular level of glutamate, glutathione, pyruvate, GABA and others did not change significantly between anaerobic and aerobic steady-states; yet their extracellular level significantly changed in response to oxygen, which is also difficult to explain based on intracellular metabolic overflow concept. In contrast, the intracellular levels of some metabolites decreased in response to aerobiosis without affecting their extracellular levels, showing that not always intracellular changes are reflected in the extracellular medium. The secretion of many metabolites cannot be explained by the metabolic overflow theory. New mechanisms must be investigated further.

POSTER 292

Comparison of global metabolite profiles of microorganisms on complex vs. minimal media

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Metabolomics shows changes in holistic views of metabolisms which are caused by genetic or environmental changes. In the study using microorganisms, medium is one of the most important environmental factors affecting the metabolism of an organism. However, there is no systematic evaluation on how the selection of complex or minimal medium can affect metabolome analysis. In this study, we have compared the global metabolite profiles of microorganisms grown on complex media and minimal media. Escherichia coli grown on Luria-Bertani medium (LB) and M9 minimal medium (M9) as rich and minimal media, respectively, were compared for their metabolite profiles. Saccharomyces cerevisiae grown on Yeast extract-Peptone-Dextrose medium (YPD) and Yeast Nitrogen Base medium (YNB) as rich and minimal media, respectively, were also compared for the metabolite profiles. The metabolic profiling was conducted by gas chromatography/time-of-flight mass spectrometry with univariate and multivariate statistical analyses. We identified 119 metabolites from these samples. They were classified into various classes such as amines, amino acids, fatty acids, organic acids, sugars and sugar alcohols, phosphates, and others. The PCA plot showed that the metabolite profiles were clearly different between the minimal and rich media regardless of strains. To investigate how the medium affects the metabolisms, we compared the sum of normalized metabolite intensities which were categorized into chemical classes and the major metabolic pathways depending on media. In this study, the class of amino acid showed higher abundance on rich media (i.e., LB for E. coli and YPD for S. cerevisiae). The classes of sugars and sugar alcohols and fatty acids were more abundant on minimal media (i.e., M9 for E. coli, YNB for S. cerevisiae). The metabolic intermediates in the synthetic pathway of various amino acids were significantly more abundant on complex media. However, the metabolic intermediates of the various sugars and sugar alcohols and fatty acids pathways were more abundant on minimal media. These study demonstrated that the microbial metabolite profiles can significantly differ depending on whether complex or minimal media are used.

POSTER 293

Metabolomics investigation of Geobacter sulfurreducens during a scale-up process

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This study is focused on optimisation of upscaling of G. sulfurreducens biomass production, to be used for the production of biogenic magnetite nanoparticles, from 100 mL bench scale to 5 L fermenters. Although upscaling of the entire process was achieved successfully and size distribution and functionality of the biomagnetite nanoparticles were comparable at both culturing scales (100 mL and 5 L), cells grown in the 5 L fermenter displayed a prolonged lag phase of ~ 24 h. With this extension of the incubation period potentially making the process inefficacious and less commercially attractive. Thus metabolomic approaches were employed for identification of growth limiting metabolites to support, aid, and potentially improve the industrial scale-up process. G. sulfurreducens strain PCA was cultivated under anoxic conditions as batch culture at 30 °C in a modified fresh water medium (NBAF) in 100 mL serum bottles and a 5 L bioreactor. Sample aliquots were taken at 13 separate time points for various analyses. Optical density (OD600nm) of samples was recorded to monitor growth behaviour of cells under the different culturing conditions. Fourier transform infrared (FT-IR) spectroscopy and gas chromatography mass spectrometry (GC-MS) were employed as metabolic fingerprinting and profiling approaches respectively, combined with multivariate statistical analysis strategies for identification of significant metabolites. During industrial scale-up of bioprocesses it is important to establish that the biological system has not changed significantly when moving from small laboratory-scale shake flasks or culturing bottles to an industrially relevant production level. In this series of experiments the goal was to assess the changes in growth behaviour and metabolism of G. sulfurreducens cells associated with the scale-up of biomass production step through monitoring growth (OD measurements), measuring metabolic fingerprints using FT-IR spectroscopy, and generating metabolic profiles using GC-MS. FT-IR results clearly differentiated between the phenotypic changes associated with different growth phases. Further, clustering patterns displayed by multivariate statistical analysis were in agreement with turbidimetric measurements, which displayed an extended lag phase for cells grown in a 5 L bioreactor (24 h), compared to those grown in 100 mL serum bottles (6 h). The collected GC-MS profiles of the cells were examined by canonical correlation analysis (CCA) to identify any significant changes in metabolites (accumulation or depletion) during the incubation period. These metabolites were overlaid onto the metabolic map of G. sulfurreducens, and the results pointed towards the limited availability of oxaloacetate and nicotinamide during the lag phase in Geobacter cells grown in the 5 L fermenter. Additional metabolite-feeding experiments were carried out to validate the above hypothesis. Although nicotinamide supplementation (1 mM) did not display any significant effects on the lag phase of G. sulfurreducens grown in the 100 mL serum bottles, it significantly improved the growth of cells in the 5 L bioreactor by reducing the lag phase from 24 h to 6 h, as well as providing higher yield in comparison to the 100 mL serum bottles. This study clearly demonstrates the application and utility of metabolomics for better understanding and improvement of industrially relevant bioprocesses.

POSTER 294

Mummichog 2: pathway and network analysis for targeted and untargeted metabolomics

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It is a major challenge to interpret metabolomics data in the context of metabolic pathways and networks. We have previously published a set of algorithms that predict pathway and network activities from untargeted metabolomics data (Li et al. 2013, PLoS Computational Biology, 9(7), e1003123). The software, mummichog, combines computational prediction of metabolites with connection patterns in metabolic networks to prioritize biological hypotheses. Therefore, it greatly accelerates scientific discovery by shifting the step of metabolite identification posterior to hypotheses generation. To meet the increasing user demands, we plan to release mummichog version 2 at this conference, which incorporates a number of improvements, including the support for targeted metabolomics data. Mummichog 2 is a Python package that runs on all major operating systems. The code will be released under an open source license. Metabolic models can be flexibly plugged into the software. The package includes metabolic data from KEGG, BioCyc, EHMN and RECON 2, and supports multiple species.

Besides enrichment tests, empirical statistical significance is assessed by permutations. The software output reports the enrichment of metabolic pathways, significant network modules and metabolites of high interest, together with a summary webpage. We will report the release of Mummichog 2, a software package that performs pathway and network analysis for both targeted and untargeted metabolomics. Mummichog is a major effort to bridge high-throughput metabolomics with genome-scale metabolic models. From untargeted metabolomics data, the software will compute probable metabolites and adducts from m/z values, and seek statistically significant patterns in metabolic reactions and pathways. This new version also includes the use of chromatographic retention time and chemical formula to improve predictions. From targeted data, enrichment on known metabolic pathways and networks will be tested. Our software program has supported a large number of both published and ongoing studies. We will demonstrate its applications to epidemiology, disease studies, environmental exposures and the identification of molecular mechanisms. First software to support pathway and network analysis for both targeted and untargeted metabolomics.

POSTER 295

Pathway-Based Analysis of Metabolomics Data in BioCyc

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BioCyc.org is a web portal containing genome and metabolic pathway databases for 5,500 organisms including Humans, Escherichia coli, Bacillus subtilis, Saccharomyces cerevisiae, and Arabidopsis thaliana. The BioCyc databases and analysis software permit metabolomics data to be connected to genome data, and provide a pathway-based framework for analysis of metabolomics data. BioCyc analysis operations include metabolite search, visualization of metabolomics data on individual pathway diagrams and on organism-specific metabolic map diagrams, and enrichment analysis of metabolomics data. BioCyc SmartTables allow users to store and analyze metabolite lists within the BioCyc website. BioCyc databases are generated computationally from the sequenced genome of the organism. Central to that process is the MetaCyc database, a metabolic describing all domains of life whose contents are derived from 43,000 publications. MetaCyc contains 2,200 pathways, 12,000 reactions, and 11,600 metabolites. MetaCyc also contains computed atom mappings for its reactions. By computationally matching enzymes in the sequenced genome against enzymes and reactions in MetaCyc, the PathoLogic software predicts the metabolic reactions, pathways, and metabolites present in the sequenced organism. The Pathway Tools softare can then automatically generate metabolic pathway diagrams, and an interactive zoomable metabolic map diagram, for the organism. Some BioCyc databases have undergone additional manual curation from the life-sciences literature. A sample metabolomics analysis workflow using BioCyc is as follows. The user identifies the metabolites in their sample using an external software package, and obtains chemical names and/or identifiers for the metabolites. They then import the file of names and/or identifiers into a BioCyc SmartTable. The SmartTable is displayed on the BioCyc website using a spreadsheet-like view in which users can select the presence of columns such as the chemical structure, molecular weight, InChI string, and identifiers for the metabolites in other databases. The metabolite set within the SmartTable can be visualized on a custom zoomable metabolic map diagram for the organism to view the metabolites within the context of the organism's full metabolic network. Metabolite concentrations are mapped to a color scale that is used to color the metabolites highlighted on the diagram. If time-series data are available, they can be viewed as an animation on the diagram. The user can also display graphs of the time courses of individual metabolites.The SmartTable metabolite set can be subject to an enrichment analysis that determines statistically whether metabolites within certain metabolic pathways are over-represented within the metabolite set.These metabolic-map displays can integrate multiple types of omics data, such as combining gene-expression and metabolomics data. Many of the preceding operations are available via BioCyc web services that allow users to submit data for visualization and analysis programmatically.The Pathway Tools software behind BioCyc supports the generation of quantitative metabolic flux models from a BioCyc database. These steady-state models predict the reaction fluxes active under a given growth condition for the organism, and can provide additional insights into metabolomics datasets. BioCyc provides unique visualization and modeling tools. Its extensive software tools integrate genome, metabolite, and metabolic pathway data.

POSTER 296

Shotgun metabolome profiling of a yeast knock-out library using a GC-Orbitrap mass spectrometer

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Metabolic processes generate a vast array of small molecule products that are closely linked to cellular phenotype. We describe a novel strategy to correlate gene function with metabolome composition by interrogation of over 1,000 yeast samples from 175 yeast knock-out strains. Selected yeast KO strains include orthologs of human disease-related genes and complementary reference genes spanning numerous cellular functions. Gas chromatography offers superior chromatographic reproducibility and is ideal for discovery-centric metabolomics. Metabolomic profiling of yeast extracts by GC-MS reveals 500 unique features. To permit feature mapping across all 1,000 analyses we utilize high resolution and accurate mass measurements of a novel platform comprising a GC interface with EI/CI source on a quadrupole Orbitrap mass spectrometer. Please consider for travel awards. Single gene deletion derivatives of Saccharomyces cerevisiae were obtained and grown on media with either dextrose (fermentation) or dextrose + glycerol (respiration). 1x108 yeast cells were isolated by vacuum filtration and washed with phosphate buffered saline, and immediately submerged into 2:2:1 acetonitrile/methanol/H2O pre-cooled to –20 °C and the extract was stored at -80 °C. Samples were thawed, vacuum dried, and derivatized in 1:1 pyridine/MSTFA for one hour at 37 °C. Experiments were performed on a novel GC-MS instrument comprising a GC interface with EI/CI source on a Q Exactive instrument platform. The system was operated in full scan mode with resolving powers ranging from 15,000 to 120,000 with electron ionization (EI). Deleting an individual gene from the yeast Saccharomyces cerevisiae and analyzing the resultant phenotype is a proven approach for characterizing eukaryotic gene function. However, many single-gene deletions do not have obvious effects on readily quantifiable biological phenotypes because compensatory molecular changes can restore biological homeostasis. To accelerate the functional annotation of eukaryotic genes we describe use of a novel GC-Orbitrap system for unbiased, discovery metabolomics (shotgun). Using this system we have developed a workflow that permits analysis of 35 yeast metabolomes per day. Samples were injected in split mode using a 1:10 ratio onto a low polarity coated capillary column. The GC gradient was linear, ramping from 100 °C to 320 °C in 30 minutes. This optimized pipeline facilitates high throughput metabolomic analyses with minimal user intervention. Each yeast metabolomic profile contains tens of thousands of m/z features. Features emanating from a singular chemical entity are grouped together into a “pure” EI-MS spectrum and assigned a unique identifier comprising retention time, fragmentation profile, and accurate mass. By resolving near-isobaric and co-eluting features, the routine sub-ppm mass accuracy and scan speed afforded by the GC-Orbitrap simplifies spectral deconvolution. Accurate mass fragmentation profiles, along with reproducible retention indices, enable the comparison of ~ 500 feature groups across all 1,000 metabolomic profiles with unparalleled specificity. Further, accurate mass measurements augment the traditional database spectrum searching methods permitting more, and higher confidence putative identifications. Monitoring the abundances of these molecules across a broad genetic background, we correlate genes of unknown function with metabolic pathways and disease. Shotgun metabolome profiling of 1,000 yeast knock-out strains using a novel GC-Orbitrap mass spectrometer.

POSTER 297

Differences in susceptibility to perturbation between the gut microbiome composition and metabolome in a genetically divergent mouse population

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The microbiome is gaining increasing attention due to the beneficial roles it plays in host physiology. Understanding the respective interplay between the host and the microbiome is complicated due to their respective variability. One approach is to simultaneously monitor the gut microbiome composition and the metabolome in a panel of lines from the Collaborative Cross (CC) mouse resource, a population of recombinant-inbred lines generated by randomizing the genetic diversity of 8 existing lines of inbred mice, and that contains a level of genetic and phenotypic diversity comparable to the human population. We characterized the gut microbiomes and metabolomes of 96 samples collected from 24 CC lines at the U North Carolina (UNC) and Lawrence Berkeley National Laboratory (LBNL). At UNC, each line of CC mice was housed in either a specific pathogen free or an ultra clean barrier facility. After transfer to LBNL, all mice were maintained in one room of a specific pathogen free facility. At both facilities, mice were maintained with free access to local water and feed (UNC, Purina 3500; LBNL, Purina 5053). Fecal samples were collected from 24 lines of the CC mice at UNC and 8 weeks after transfer to LBNL and were subsequently frozen in liquid nitrogen. Samples were homogenized and split into two fractions for 16S sequencing and metabolomics, respectively. Metabolites were extracted using a methanol/sonication method and were dried in vacuo. Dried metabolites were chemically derivatized and analyzed by GC-MS. We identified a total of 122 metabolites from the 96 fecal samples after matching the GC-MS-based metabolomics data to an in house database containing mass spectral and retention index information for over 850 entries. An additional 110 peaks were detected but not identified. We observed the most significant clustering of the metabolomics data by institution: LBNL samples clustered distinctly from UNC samples. Clustering was also generally observed for different mouse lines suggesting genetic variation and interaction of genetic variation with environment. Other factors such as gender and facility at UNC did not drive clustering of the metabolome. In contrast, preliminary 16S sequencing showed that the microbiome strongly clustered based on the facility in which the mice were raised at UNC. Thus, the host environment established a microbiome structure which persisted through life in contrast to the metabolome. We will show how individual metabolites and metabolic processes changed, and also discuss how metabolomics data can be integrated and interpreted with 16S microbiome data from the same samples. In addition, we will present data that assesses the reproducibility of our approach for measuring the metabolome in mouse feces. Our research indicates that the interaction between host genetics and environment significantly influences the fecal metabolome and microbiome.

POSTER 298

The effect of TCA cycle loss of functions on the metabolic profiles of liver specific knockout.

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The tricarboxylic acid (TCA) cycle is an essential mitochondrial pathway in liver that captures energy by oxidation of acetyl-CoA and supplies building blocks for biosynthesis by high capacity anaplerosis and cataplerosis. These three functions are elegantly balanced to ensure appropriate substrate and energy supply for downstream biosynthesis and a constant level of intermediates for TCA cycle function. Dysregulation of these pathways are components of many diseases including diabetes and cancer. Yet because of the cyclic nature of these pathways, insight based on metabolite concentrations can be difficult to link to actual function. Therefore, we used NMR spectroscopy to profile the individual effects of loss of hepatic anaplerosis, cataplerosis and oxidative function of the TCA cycle in knockout mice. Three mouse models developed by liver specific knockout of one of key anaplerotic/cataplerotic/TCA cycle enzyme were studied. Six cohorts of mice were subjected to tissue collection (three knockout - KO and three corresponding wild types - WT). Liver from overnight fasted mice were snap frozen in situ. Frozen liver tissue was pulverized and extracted by MeOH/H2O/CHCl3. The polar phase was collected, evaporated to dryness, reconstituted in phosphate buffer and examined by 1H NMR spectrometry at 600 MHz. Metabolites were confirmed using 2D NMR and standard compounds mixtures. NMR signals were analyzed using unsupervised PCA and supervised OPLS-DA models. Assignments of NMR signals identified 30 metabolites in liver polar extracts. PCA analysis revealed distinct sources metabolic variability depending on whether anaplerosis, cataplerosis or oxidative function was genetically reduced. Differences between KO and WT livers were observed in all three mouse models. Subsequent discriminant modeling using OPLS-DA resulted in complete separation between KO and WT groups and allowed identification of metabolites with highest contribution to discrimination. Loss of cataplerosis - Loss of cataplerosis in liver resulted in significantly diminished glucose concentration in liver. In contrast, the levels of TCA cycle intermediates were significantly increased, as were glutamine, glutamate and aspartate which are in exchange with the TCA cycle. These results are consistent with the loss of a gluconeogenic pathway that consumes TCA cycle intermediates. Loss of anaplerosis - Elimination of key anaplerotic enzyme decreased amino acids that supply TCA cycle such as glutamine, glutamate and aspartate. In contrast to loss of cataplerosis, loss of anaplerosis had the biggest effect on metabolites not directly in the TCA cycle. Increased levels of citruline, lysine and ornithine suggest interplay between nitrogen metabolism and the anaplerotic function of the TCA cycle. Loss of oxidative span of the TCA cycle - Finally, loss of a TCA cycle enzyme required for the turnover of the cycle resulted in reduced glucose, but there was surprisingly little perturbation of TCA cycle intermediates. Thus, the TCA cycle is required for gluconeogenesis but there may also be compensatory pathways that allow the cycle to proceed even when an important step is removed. We conclude that changes in the metabolic profile of the TCA cycle can be caused by defects in anaplerosis, cataplerosis and/or its oxidative function. Careful consideration of the downstream and upstream metabolites need to be considered when interpreting the metabolic profile of the TCA cycle. The livers of mice with anaplerosis, cataplerosis and oxidative TCA cycle loss of function were studied using NMR metabolic profiling.

POSTER 299

Examination of the global yeast metabolome impacted by the deletion of lipin phosphatidate phosphatase (Pah1p), using IROA and GC-TOF-MS

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The yeast metabolome has metabolic pathways common to all eukaryotes. Lipin is a phosphatidate

phosphatase which can convert phosphatidate to diacylglycerol, making it a key node for energy storage via the production of triglycerides (PA->DAG->TAG). Mammalian lipin absence results in lipodystrophy, and yeast lipin (Pah1p) controls the formation of cytosolic lipid droplets. A yeast deletion of Pah1p (PAH1Δ) has been shown to result in a dramatic decrease in lipid droplet number. Here, we used a GC-TOF MS with Isotopic Ratio Outlier Analysis (IROA) technology to examine the metabolome changes of Pah1p deleted yeast. IROA is a mass spectrometry-based metabolomics profiling method using 13C labeling to eliminate sample-to-sample variance, discriminate against noise and artifacts, and improve identification of compounds. Wild-type and pah1Δ::natR CEN.PK yeast strains were grown to mid log phase on Yeast Nitrogen Base without amino acids, containing 5% or 95%13C glucose. Yeast metabolites were extracted after harvesting at -20° C in 4:4:2 (v:v:v) methanol:acetonitrile:water. Extracts were analyzed at Einstein using a Waters GC-TOF-MS after methoximation/BSTFA derivatization, for electron ionization (EI) and chemical ionization (CI), using a DB-5MS column (30×0.25 mm, 0.25 µm). An alkane (C8-C40) retention time index mixture was used. 95% methane/5% ammonia was used as the CI regent gas. IROA peak pairs were identified for EI and CI spectra using Genedata Expressionist MS Refiner software. Validation for IROA CI spectra using Cluster Finder (IROA Technologies) software was done at the Southeast Center for Integrated Metabolomics. Normally the natural abundance of 13C in metabolites is low. With IROA substrates, containing 5% or 95%13C glucose, the 13C metabolite abundance response is enhanced to form characteristic mirrored peak pairs of 12C M and 12C M+1, and 13C M and 13C M-1 peaks. A total of 107 clusters in the EI mode, and 133 clusters in the CI mode, showed IROA peak pairs with 12C M and M+1peaks mirrored by 13C M and M-1 peaks for the same deconvoluted cluster. Approximately 60% of EI clusters could be found in Fiehn, NIST and in house GC/MS databases, and these were used to validate IROA identities. For example, citrate or ornithine, were co-eluted in one cluster, detected as the IROA peak pairs of 12C- m/z142/13C-m/z 146 and 12C-m/z 273/13C-m/z 278 in the EI mode. However, the identities could be discerned as the IROA CI peak pairs of 12C-m/z 421/13C-m/z 426 (ornithine) and 12C-m/z 481/13C-m/z 487 (citrate). For the unknown peaks, formulae for chemical identities were determined, using the exact mass isotopomers and IROA m/z peak pair mirrors. The IROA CI molecular ion m/z peak pair mirrors the difference between the unlabeled 12C M0 peak and the fully labeled 13C Mn peak by the number of N carbons in the metabolite which allows the deduction of chemical formulae for the unknowns. Using IROA, it was found that glycolysis is enhanced in the yeast pah1p deletion vs WT. The metabolite response showed increased glucose-6-P, fructose-6-P, and glycerol-phosphate. Alterations were seen in lipid and steroid metabolites (increased phosphoethanolamine, palmitoleate, oleate, lanosterol and dihydrolanosterol), as well as in the TCA cycle (increased citrate and decreased fumarate). The results also suggested alterations in amino acid metabolism (decreased orthinine and citrulline). First description of global yeast metabolome using IROA and GC-TOF-MS to examine metabolic differences, here assessing lipodystrophy, using yeast PAH1Δ.

POSTER 300

Metabolomics-derived insights into the effect of methyl-jasmonate on secondary metabolite pathways in Centella asiatica cells

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Centella asiatica is an important source of pentacyclic triterpenoid secondary metabolites (centellosides), associated with a spectrum of biological activities and the enhancement of their biosynthesis is receiving much attention. Elicitor action by exogenous methyl jasmonate (MeJA), leads to dynamic metabolic changes that involve alterations in some steps of the terpenoid pathway, with an increase in triterpenoid levels. However, other less well characterized metabolites such as chlorogenic acids and sesquiterpenoids also contribute to the reported biological activities. To further interrogate the MeJA-induced metabolic changes in C. asiatica cells we employed a metabolomic approach. Metabolomic analyses can provide a more detailed and comprehensive metabolic picture under specific conditions and contribute to the understanding of the cellular responsiveness. Cultured cells of C. asiatica in suspension were elicited with 100, 200, 400 µM MeJA and incubated for 24 h. Extracts were prepared with methanol

for a broad-based polar metabolite profile and also with a chloroform-based dispersive liquid-liquid micro extraction (DLLME) procedure for extraction of non-polar metabolites. GC-MS and UHPLC-MS platforms were employed for semi-targeted and untargeted analyses, respectively. Masslynx and Profiling Solutions software were used for UHPLC-MS and GC-MS data processing respectively. Multivariate data analyses were performed utilizing SIMCA and XCMS software. Results indicate concentration-dependent changes in the metabolite profiles of control and MeJA treated cells, indicative of the cellular response to MeJA. The annotated ions (statistically significant/discriminating m/z) exhibited a positive or negative correlation (uPoster or down-regulated) to the MeJA treatment of the C. asiatica cells. These metabolites indicated a MeJA-induced alteration in different metabolic pathways, including flavonoid-, phenylpropanoid (cinnamates)- and terpenoid pathways and changes in fatty acid profiles. MeJA treatment triggered the accumulation of bicyclic sesquiterpenoids (aristolochene, deoxy-capsidiol, 15-hydroxysolavetivone, solavetivone, 3-hydroxylubimin) and a tricyclic sesquiterpenoid (phytuberin). These compounds are of special interest as they are stress-related secondary metabolites with antimicrobial activities (phytoalexins) reported in potato and tobacco plants. These sesquiterpenes are not found in healthy or control tissues, but only accumulate in response to elicitation or pathogen challenge. These observations raises the interesting question of constitutive (phytoanticipin) vs. inducible (phytoalexin) sesquiterpenoids in C. asiatica and the enzymes involved in their synthesis: sesquiterpene cyclases (SQCs) or 2-trans, 6-trans-farnesyl-diphosphate diphosphate-lyases. SQCs can form several cyclized products (e.g. cyclizing, aristolochene-forming) and there exists a correlation between specific amino acid motifs / active site sequences in SQCs and the cyclization type. In contrast to the sesquiterpenoids, flavonoids were mostly negatively correlated with the treatment. The presence of the bioactive sesquiterpenoids in MeJA-elicited cells and other tentatively identified metabolites (abscisic acid, fatty acids, phytosterols and metabolites of shikimate-phenylpropanoid pathways) indicates that the changes in the metabolome are associated with a defensive function in response to elicitation by MeJA, rather than just the amplification of existing terpene pathways. New insights into flexible and controllable aspects of metabolic manipulation of C. asiatica cells by MeJa.

POSTER 301

Whale: a package combining fingerprinting and targeted metabolite profiling to improve the extraction of metabolic information in NMR spectra.

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An NMR spectrum is composed by resonances of a huge number of metabolites; and obtaining its metabolic profile can provide very valuable biological information. Depending on the final goal, different approaches can be applied to analyze the samples in a dataset. An untargeted analysis of the sample fingerprint can be enough to discriminate groups in a study, or very useful for finding 'hot spots' in an exploratory analysis. Otherwise, a targeted analysis of the metabolite profile can be performed in order to find molecular patterns of the samples under study. We present a package that allows the user to use both approaches and to include unknown signals and 2D acquisitions for an exhaustive analysis of the NMR data. The package works under an intuitive and user-friendly GUI and has default libraries and default sets of deconvolution patterns easily editable by the user. All libraries and patterns were built using public and private databases and the know-how acquired through the analysis of numerous datasets of different biological matrices. It is equipped with functions that automate fingerprinting techniques such as spectral correlations between both spectral regions and metadata and 'hot-spots' detection through spectral variance between samples. It offers automatic quantification approaches that can be tested before being applied to the whole dataset and it also incorporates several visualization and editing options that allows the user to actively interact with the samples, avoiding black-box processes but giving automatisms to minimize subjectivities. We used the package to analyze several datasets of different biological matrices previously studied using manual methods of spectra visualization (using

TopSpin) and metabolite profiling (using Chenomx). When comparing visualization methods of established packages against ours is important to highlight some useful aspects in behalf of our package. Visualization of the mean and the median spectra can provide information about the spectral alignment, and visualization of the 'hot spots' spectra allows the user to focus the analysis (at least as starting point) in regions of the spectra that present major changes between groups or that correlate with metadata parameters. For a targeted analysis of the metabolites present in a sample our tool offers signal suggestions in the regions of interest (ROI) selected by the user. Moreover, the user has de chance to import 2D acquisitions such as JRES, COSY and HSQC and to compare the spectra with a library of standard compounds. To quantify the selected metabolites, the user can choose between 3 different methods of signal quantification; area integration, clean fitting and baseline fitting, depending on the experiment necessities, which allows optimizing processing times. Finally, all the quantification iterations that do not pass a quality threshold based on the fitting error and signal to noise ratio will be saved in a folder, giving the chance to the user to discard or re-adjust the fitting parameters in order to finally obtain the most reliable results. All this workflow has improved the time span of NMR data processing in all the studies previously analyzed with the well established methods. The same biological conclusions were achieved while user subjectivities were reduced, and focusing the target analysis to the 'hot spot' spectral regions saved users to work with noisy data. This study presents a complete package for NMR data processing which combines the benefits of untargeted and targeted analysis.

POSTER 302

A novel algorithm for automated untargeted metabolite discovery for high-throughput 1H-NMR metabolomics

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NMR is one of the major metabolomics acquisition platforms. It provides highly reproducible and resolved spectra in a short period of time, which makes it very suitable for clinical diagnostics. However, the analysis of NMR data is cumbersome, operator-biased and time consuming, because the spectra contain thousands of resonances that may belong to hundreds of metabolites. The major challenges are overlapping spectral components, multiplets of signals, noise, and an unknown number of metabolites in the sample. To overcome these challenges, we present a new powerful method for automated identification of both ‘knowns’ and ‘unknowns’ in a given sample. SPatial clustering Algorithm coupled to Statistical Total Correlation Spectroscopy (SPA-STOCSY) thus provides a complete analytical tool for the metabolomic NMR data analysis. As a data driven method, SPA-STOCSY estimates all parameters from the input dataset. The underlying principle of SPA is based on the strong dependencies of signals from the same metabolite. SPA identifies such patterns by investigating the covariance structure of the input dataset. A moving average approach scans the average pair-wise correlation among adjacent data points in a window of estimated size. Based on this average correlation landscape, an unsupervised machine learning approach automatically determines the optimal threshold necessary to group data points with strong dependencies into clusters. We then use STOCSY to identify highly correlated clusters, presumed to belong to a metabolite. These are then automatically linked to a chemical library to identify the structure and name the metabolite. We tested SPA-STOCSY performance on both synthesized and real NMR spectra to ascertain efficiency and accuracy of the method. The ideal method should identify clusters that include majority of the signal regions while avoiding noise regions. True coverage rate measures how well the identified clusters capture true signal regions and noise coverage rate measures the coverage of noise signals in the identified clusters. Further, if there is an overlap of signals belonging to two metabolites, the clusters should split for better discrimination of overlapping signals. We designed 12 simulation scenarios varying the total number of metabolites in the sample, the noise level, the

metabolite concentrations, and sample sizes. In each simulation scenario, we randomly generate 100 datasets, each containing at least 50 synthesized spectra. Using synthesized spectra, SPA clusters achieved high true coverage rate (median value=0.6-0.8), while noise coverage rate was <0.01. In contrast, Statistical Recoupling of Variables (SRV), also based on clustering, performed at 0.4-0.6 and 0.15-0.3, respectively. Thus, the SPA performed significantly better than the published SRV (p<0.0001). To then identify metabolites in the spectra, we chart the correlations among clusters (instead of variables, as commonly employed) using STOCSY linked to a library of 384 metabolites. SPA-STOCSY successfully identified 9 out of 10 metabolites from spectra where most metabolites had overlapping signals. Using real 1H-NMR data obtained from the brain homogenates of Drosophila melanogaster (N=10 samples, 40 brains per sample; spectra acquired using 800MHz NMR, Varian), SPA-STOCSY identified 85 metabolites and predicted 7 that were not in the library, with total computation time <7 minutes. Compared to operator-based Chenomx analysis, SPA-STOCSY matched 84% of the identified metabolites. Overall, as a fast and automatic tool with high accuracy, SPA-STOCSY is a valuable un-biased tool for professionals either in or outside cheminformatics field for untargeted compound identification and discovery. SPA-STOCSY is a unique, automatic, fast, accurate and reproducible method for discovery of both 'known' and 'unknown' metabolites.

POSTER 303

Novel analytical methods and large-scale data analysis strategies for metabolomic analysis of Leishmania donovani

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As part of a large-scale project in the R&D of anti-leishmanial compounds, whereby academia meets industry through an 'open lab' collaboration; novel analytical and data strategy methods are under development. The first involves the development of a novel metabolomics protocol to obtain the optimal profile of Leishmania donovani axenic amastigote parasites. In the second phase of method development for this large-scale project, novel strategies in data treatment are under development. One theme involves a novel strategy for the treatment of missing values in large-scale metabolomics data to improve robustness of data treatment in such experiments. The axenic amastigote form of Leishmania donovani has never been analysed by multiplatform metabolomics utilising LC-MS, GC-MS and CE-MS. We developed a metabolomics method to analyse a single sample (validated with replicates) across all platforms in order to gain the widest coverage of the metabolome. Method development focussed on sample preparation as well as optimisation of the analytical methods. Missing value imputation strategies were investigated using plasma as a known model. Four methods were compared (zero, ½ minimum, median, kNN) to reveal their effects on data normality/variance, on statistical significance and on the approximation of a suitable threshold to accept missing data as zero. Additionally, different strategies for FDR correction were investigated with respect to data after missing value imputation. A robust method for sample preparation has been developed that is applicable to all platforms analysing a single sample. The optimal number of parasites required in a single sample, enough to analyse in all three techniques was 8×107. Best extraction of metabolites was observed using methanol, in which parasites were mechanically broken using a tissue lyser and freeze-thaw cycling in liquid nitrogen. Analytical method development enabled profiles to be obtained for global fingerprinting by LC-MS, GC-MS and CE-MS in addition to lipid fingerprinting by LC-MS. Combining the profiles from each technique, a wide variety of metabolites and lipids were able to be detected in a reproducible way. This shows excellent promise for the large scale study in which up to 50 compounds with anti-leishmanial activity from GSK's 'Leishmania box' will be compared through this developed metabolomics approach. From the missing value imputation investigation, it was observed that normality and variance of data are affected by missing values and kNN imputation was the best tested method for restoring this. Bonferroni

correction was the best method for maximising true positives and minimising false positives and it was observed that as low as 40% missing data could be truly missing and should be imputed by zero rather than any other value. The range between 40 and 70% missing values was defined as a 'grey area' and therefore a novel strategy has been proposed that provides a balance between the optimal imputation strategy that was kNN and the best approximation of positioning real zeros. Method development for multi-platform metabolomics analysis of Leishmania donovani amastigotes; novel strategy for missing value imputation in large-scale metabolomics data.

POSTER 305

Leveraging multiple disparate datasets: applying novel visualization and statistical tools to a fecal microbiome study

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In order to achieve a more holistic understanding of the fecal microbiome in the context of bariatric surgery, we are developing methods to integrate and interrogate multiple disparate data types. Using statistical tools including trelliscope and datadr, we are able to visualize composite data and explore biomarker relationships. Our goal is advance understanding of microbiome conditions related to energy regulation and patient responses to the most commonly performed bariatric surgery in the United States, the Roux-en-Y gastric bypass (RYGB). We seek 1) to determine the relationship between the patient fecal microbiota and success of RYGB and 2) to identify biomarkers or clusters of biomarkers that relate the composition of the fecal microbiota to patient health status. Our study included subjects from four distinct groups: obese, normal weight, successful and unsuccessful RYGB (unable to maintain loss of 50% or more of excess body weight). From these groups, we have access to five different data types, including 16S rDNA sequences, NMR, GC-MS- and LC-MS-based metabolomics, and patient metadata (dietary intake). Such disparate measurements are seldom statistically analyzed simultaneously. We present statistical methods for conducting efficient exploratory data analysis using a divide and recombine methodology for big data through datadr and visualization using trelliscope in R statistical software. Feature selection is performed on combinations of the multiple available data types using support vector machine (SVM) algorithms with variable importance scores and linear discriminant analysis (LDA) with variable selection. We have thus far identified several types of data that show statistical ability to discriminate patient groups. In the patient metadata category, "average carbohydrate consumption" showed a general discriminatory ability, and "average alcohol consumption" was able to discriminate successful versus unsuccessful surgeries. In examining groupings of metabolites, "mono- and disaccharides", "amino acids" and "aromatic compounds" were useful in general discrimination of groups, and "tryptophan metabolism" and "food components" were able to differentiate successful and unsuccessful surgeries. Looking at individual metabolites, N-acetylglycine, glucose, lysine and tryptophan showed general discriminatory capabilities, and glycolate, isobutyrate, isovalerate, N-acetylglycine and tryptophan were able to discriminate successful and unsuccessful surgeries. When examining 16S rDNA data, Enterococcus, Klebsiella, Rothia, Slackia, Streptococcus, Trabulsiella, and Veillonella possessed general discriminatory ability, and Blautia, Coprobacillus, Mogibacterium, Pseudomonas, and Pyramidobacter were able to discriminate successful from unsuccessful surgeries. Furthermore, feature selection performed on all data sources simultaneously provides significant improvement over any of the

single data sources' capability to discriminate between groups of interest. Our research employs novel tools and statistical methods to integrate and interrogate five distinct data types.

POSTER 306

Variable selection for binary classification using error rate Poster values applied to metabolomics data

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Metabolomics datasets are high-dimensional though only a limited number of variables are important for binary discrimination making identifying discriminatory variables an important yet complex task. Traditional statistical methods make unrealistic assumptions when applied to metabolomics. Non-negative measurements violate normality assumptions required by parametric methods (e.g. t-test). Nonparametric equivalents lack sufficient power when group sizes become small (e.g. Mann-Whitney test). Most methods are sensitive to unequal group sizes (e.g. logistic regression), a limitation when investigations require invasive procedures. More robust methods are generally sophisticated (e.g. PLS-DA), and difficult to implement by insufficient experienced metabolomics investigators. Here we introduce a novel and less-sophisticated approach to variable selection, ERp (Error rate Poster values) and illustrate how it can be used to classify new cases. We assume that the experimental group tends to have either higher or lower observed values compared to the control group. Shifted metabolites then have some threshold value which can be used to discriminate between the two groups. Classification error rates are related to the choice of threshold and small error rates indicate important discriminators. But how small must the error rates be for the associated metabolite to be important? We answer this question by making use of non-parametric hypothesis testing based on error rates. This helps to answer the question in terms of the familiar concept of Poster values. Once statistically significantly shifted metabolites have been identified, the threshold corresponding to the error rate can be used to classify new subjects. Our main contribution is to show that non-parametric hypothesis testing, based on minimum error rates, can identify statistically significantly shifted metabolites. We find that the discriminatory ability of variables becomes more apparent when error rates are evaluated based on their corresponding Poster value as relatively high error rates (0.25) are still significant. ERp can accommodate unequal and small group sizes while accounting for the cost of misclassification into either group. ERp retains (if known) or reveals (if unknown) the shift direction, aiding biological interpretation. The thresholds resulting in the minimum error rates can be used to classify new subjects. We subsequently illustrate ERp on NMR-generated metabolomics data (Moutloatse et al, 2015). The variables selected based on a combination of univariate (Mann-Whitney Poster values and effect sizes) and multivariate (PCA Power and PLS-DA VIP values) methods are similar to those flagged by our single method. Furthermore, when variable lists are presented for biological interpretation, all relevant variables are present, with appropriate shift directions, in our single list. To ensure robust error rate and threshold estimation, we also investigate LOO (Leave-One-Out) estimates. We find that LOO estimates of error rates are less powerful which is of interest since this is counterintuitive. Also, LOO threshold estimates and the estimate obtained using all cases are very comparable in terms of bias. These conclusions are based on ten different simulation scenarios repeated ten thousand times. Therefore, the final approach does not require such a cross-validation procedure making it even less time-consuming. Finally, we illustrate how estimated threshold of selected variables can be used to classify new cases. Future research will investigate the impact of large proportion of observations below the detection limit ERp performs variable selection and classification, is non-parametric, robust to unequal groups, accounts for misclassification costs and aids biological interpretation.

POSTER 307

A data-driven approach to identify discriminatory biomarkers from proton nuclear magnetic (NMR) resonance data

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In an attempt to identify potential discriminatory markers in metabolic profiling studies, a reliable method for biomarker selection process is of paramount importance. At presence, the biomarker selection process is not optimized and is user-dependent. Here, we propose a novel data-driven framework for extracting biomarker information from spectroscopic data. Our proposed data-driven method, known as Biomarker Information Recovery by Statistical spectroscopY (BIRSY), uses statistical characteristics of each spectral variable and clustering method, to cluster the spectral variables into three groups: spectral variables lacking any metabolic structured information (i.e., noise); non-discriminatory metabolites; and discriminatory metabolic biomarkers. To evaluate our proposed algorithm, a simulated proton NMR spectral dataset (N= 500 in each group) was generated to emulate toxic effect of a model nephrotoxin, Paraquat and control group. The Paraquat toxicity group showed higher signal intensity for lactate and L-alanine; and lower intensities for creatinine and citrate when compared to the control group. We compared the performance of our method in identifying genuine biomarkers to univariate t-test, after adjusting for Bonferroni correction. Orthogonal Partial Least Squares Discriminatory Analysis (OPLSDA) comparison between the Paraquat toxicity and the control group showed a model Q2 statistics of 0.85, P 0.67 as metabolic biomarkers. Based on these criteria, we found both methods correctly identified all four metabolic biomarkers. Both the specificity and positive predictive value (PPV) for BIRSY were 100%. This demonstrated BIRSY approach did not show any false positive result. This result was superior to univariate approach, with a specificity and PPV of 95% and 29%, respectively. The lower specificity and PPV for the univariate approach was due to the incorrectly identification of 5% of the spectral variables as biomarkers. Our results clearly show that BIRSY, which does not rely on the users to pre-set criteria for discriminatory metabolites selection, but purely relies on the dataset itself, can correctly identified all genuine biomarkers with no false positives. This result is superior to the standard univariate approach, which is often deemed to be too stringent. BIRSY can automatically identify all genuine biomarkers in the metabolic profiles with no false positive and outperforms standard univariate approaches.

POSTER 308

eRah: an R package for automatic spectra deconvolution, alignment, and library matching of metabolites from GC/TOF-MS data in untargeted metabolomics

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GC/MS-based metabolomics produce large and complex datasets characterized by co-eluting compounds and fragmentation of molecular ions caused by the hard electron ionization (EI). Free and open-source integrated worksflows allowing for the identification and quantification of the corresponding metabolites are urgently needed. Here we introduce eRah, an R package that allows for an innovative deconvolution of co-eluting compounds using multivariate analysis techniques, alignment of spectra across samples, and automatic identification of metabolites by spectral library matching. eRah outputs a table with compound names, matching scores and the area of the compound for each sample. We demonstrate the functionality of eRah with a comparative analysis of plasma samples of adolescents with hyperinsulinaemic androgen excess and healthy controls. The eRah package has been designed in an open-structure, where different algorithms for deconvolution, alignment, identification or statistical analysis can be integrated. Here a moving chromatographic window is used to apply orthogonal signal deconvolution - a combination of independent component analysis and principal component analysis - to resolve the different compounds and further align them across multiple samples on the basis of the elution profiles and composite (i.e., pure) spectra. Next, we use a partial least squares regression to

recover those compounds that could not be resolved initially or could not be correctly aligned. Finally, identification of metabolites is achieved automatically by comparing the resolved mass spectra against a reference spectral library such as the Golm Metabolome Database. The eRah workflow was tested using 11 plasma samples of adolescents with hyperinsulinaemic androgen excess and 14 age - and weight - matched healthy controls analyzed by GC/EI-qTOF MS (Agilent Technologies). With the aim of comparing the quantitative results, the data set was processed using eRah and XCMS. The latter uses centWave, a highly sensitive peak detection algorithm. From among resolved composite spectra using eRah, lactate, myo-inositol, ornithine and urea showed statistical significant differences between disease and control groups, both through the area and the intensity of the highest peak in the composite spectra. XCMS in turn revealed different mzRT features related to these four metabolites that also presented statistical differences. Furthermore, the identity and quantitative differences (i.e., fold change and Poster value) in these metabolites were validated through targeted analysis using a GC-triple quadrupole (QqQ) MS. Altogether, eRah shows an excellent correlation XCMS and QqQ MS targeted analysis, with the manifest advantage that eRah provides a complete automatic data analysis workflow for GC/MS-based untargeted metabolomics. eRah is a complete GC-MS data workflow incorporating an innovative deconvolution process that differs from existing tools such as AMDIS.

POSTER 309

geoRge: a computational tool for stable isotope labelling detection in LC-MS untargeted metabolomics

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Studying fluxes of chemical moieties through the complex set of interacting metabolic reactions is essential to understanding the alterations in homeostasis that occur in disease. Experimental determination of these fluxes can be inferred from the characteristic rearrangement of stable isotope tracers (e.g., 13C or 15N) through metabolic pathways. This approach typically uses mass spectrometry and isotopically labelled metabolites to trace back the fate of labelled atoms into the structures of downstream metabolites in the metabolic network. LC/MS-based untargeted metabolomics can facilitate the unbiased mapping of labelled moieties, however few tools are available for data analysis. Here we introduce geoRge, a novel computational workflow to fill this gap. ARPE-19 cells were cultured under standard conditions in which they constitute a monolayer that retains the functionality, polarity and tight junction expression of the human retinal pigment epithelium. For our study, cells were seeded and maintained in culture for 21 days with 5.5 mM or 25 mM of D-glucose at 37ºC under 5% (v/v) CO2. During the last 24 hours, cells were sustained in serum deprived media with 5.5 mM or 25 mM of U-[13C]-D-Glucose or D-glucose. Metabolites were extracted using chloroform/methanol (2:1 v/v). LC/MS analyses were performed using an UHPLC coupled to an ESI-QqTOF MS operated in negative and positive ESI mode. Each condition was run in triplicate. Here we present geoRge, an R-based workflow capable of analyzing non-targeted LC/MS data from stable isotope-labelling experiments. GeoRge uses XCMS centWave algorithm to perform peak picking on data from labelled experiments and their corresponding non-labelled controls. The presence of newly emerged or greater intensity mass spectral features in the labelled samples by comparison with the non-labelled controls has been the basis for establishing geoRge. Thus, geoRge treats statistically significant uPoster regulated features in labelled samples as stable isotope-labelled metabolites. Next, the maximum number of stable isotope atoms in the labelled metabolite is estimated by dividing the m/z value of the uPoster regulated feature by the exact mass of the stable isotope (e.g., 13.00335 for 13C or 15.000108 for 15N). This information is used to find the base peak, namely the unlabelled form of the metabolite, in the matrix of features. The detected base peaks are matched automatically against the Human Metabolome Database for putative identification. To validate geoRge, we analyzed an in vitro human model of diabetic retinopathy: retinal pigment epithelial cells (ARPE-19) cultured in normoglycemic (5.5 mM D-glucose or U-[13C]-D-glucose) and hyperglycemic (25 mM D-glucose or U-[13C]-D-glucose) conditions. From all the putative incorporations, 35.8% and 58.9% were specific of normoglycemia and hyperglycemia, respectively, while 5.2% were shared in both

glucose concentrations. In order to confirm the identity of the unlabelled base peaks and verify that the labelling occurred, some of the unlabelled and labelled features were confidently identified and characterized using MS/MS experiments. Statistical comparison between unlabelled and labelled samples is a robust method for tracking stable isotopes in untargeted metabolomics.

POSTER 310

An automatic workflow for analyzing untargeted stable isotope tracking experiments to study diabetic rethinopathy by GC-TOF MS

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The use of GC-electron ionization (EI) MS to study metabolic fluxes by experimenting with stable isotope-labelled metabolites is hampered by the difficulty of analyzing the datasets derived. Here we introduce eRah, a computational tool that allows the unbiased tracing of labelled atoms through automatic spectra deconvolution, alignment, and library matching of metabolites from GC-TOF MS. To test eRah we have analyzed an in vitro model of diabetic retinopathy that simulates hyperglycaemic conditions on the metabolism of a human retinal pigment epithelial cell line (ARPE-19). ARPE-19 cell culture were maintained in culture for 21 days with 5.5 mM or 25 mM of D-Glucose at 37°C under 5% (v/v) CO2 in an incubator. Serum deprived media (24 hours) were prepared with 5.5 or 25 mM of either D-Glucose or [U-13C]-D-Glucose. Each condition was run in triplicate. Cell extraction was performed using chloroform/methanol (2:1). Aqueous phase were lyophylized, derivatized and analyzed using a 7200 GC-qTOF MS (Agilent Technologies). Human RPE cells were exposed to hyperglycaemic and normoglycaemic conditions using 25mM and 5mM [U-13C]-D-Glucose or unlabelled D-glucose, respectively. Unlabelled spectra were deconvolved using eRah through a combination of independent component analysis (ICA) and principal component analysis (PCA). Partial least squares (PLS) regression was used to fit composite (i.e., pure) spectra from non-labeled samples in 13C-labeled samples in order to retrieve the spectra of the labelled metabolites. Then each m/z of the composite spectra in non-labelled and labelled samples were compared using a t-test with the aim to identify labelled fragments. Statistically significant uPoster regulated m/z in labeled composite spectra were considered as 13C-incorporations. Finally, the identification of labelled metabolites was achieved indirectly by comparing automatically the resolved unlabeled mass spectra against a reference spectral library. GC-chemical ionization (CI)-TOF MS analyses of the same samples were used to validate our data. Our results show a characteristic rearrangement of the stable isotope tracer in hyperglycaemic conditions in metabolites of the polyol pathway. Although it has been reported in several retinal cell lines (e.g., retinal ganglion cells, Müller glia, vascular pericytes and endothelial cells) that activation of the polyol pathway by diabetes contributes to the development of diabetic retinopathy, few data on this issue exist for RPE. An automatic workflow for analyzing stable isotope tracking experiments using GC-TOF MS.

POSTER 311

Automating LC-MS and MS/MS based Large Metabolomic Data Processing and Analysis using SimMet

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Liquid chromatography- mass spectrometry (LC-MS), with high sensitivity and low amount of sample

requirement, is one of the leading analytical platforms applied for metabolite profiling [1-3]. Multiple software tools are often used to analyze such data. For example, data analysis through MetaboAnalyst [5] needs XCMS [6] processed peaklists. Besides, these cloud based tools are are associated with downsides of cloud computing [7] such as (i) Reduced control over the distribution of the computation, (ii) Data transfer problem due to network bandwidth issues, and (iii) Privacy concerns. In order to address the challenges, we have developed SimMet. The software workflow will be demonstrated based on a food metabolomics experiment with data acquired on an LC-MS QTOF system. Capsules of 13 different types of coffee were extracted using 35 ml of water on a standard coffee capsule machine. Nos. of replicates: Two and Extracts were diluted 1:50 in water prior to analyzing 5µl of 3 replicates for each extract by UHPLC-MS. QC sample: Pooled equal volumes of all the 13 coffee samples. LC Method: RSLC system (Dionex) with a 50 x 2.1 mm BEH C18, 1.7 um column (Waters) with LC gradient program: linear increase from 2% B to 98% B (over 5 min), constant at 98%B (for 1 min). MS Method: Compact Qq-TOF mass spectrometer (Bruker Daltonik); ESI +ve mode, MS full scan and auto MS/MS data acquired at a speed of 3 Hz . Software: SimMet SimMet generates peaklists of peaks detected in LC timescales for raw data in batch mode. Compound features (isotopes such as M+0, M+1 M+2,..., and adducts such as, NH4, Na, Li, K etc.) are combined thereby reducing the LC-peaks to a great extent. MS/MS scans are clustered corresponding to these compounds. Peaklists are aligned based on retention time and precursor m/z. Options to review and remove unwanted peaks, gap filling for missing peaks are also facilitated ensuring robust data matrix for accurate statistical analysis. The aligned peaklist is subjected into Principal Component Analysis. The PCA scores plot separates strong, medium and weak coffee extracts. Two analytes with m/z values 124.0394 and 138.0561 mainly contributing to the separation of samples in the PCA scores plot can be accessed from the corresponding loadings plot. Exact mass search with 5 ppm tolerance in multiple online (?) metabolite databases enabled the tentative identification of the unknown compounds on the basis of minimum delta mass. However, there are other compounds that are reported by the program with same delta mass thereby making the identification unreliable. Hence, compound identities were elucidated using the MS/MS capabilities of the Compact QTOF instrument and then by subjecting to SimMet MS/MS search module. The compound with m/z value 124.0392 was identified to be nicotinic acid while structures of picolinic acid and nitrobenzene are identified as the second and third ranked structures. The compound with m/z value 138.06 was identified to be trigonelline.

References 1. Castrillo et al. Phytochemistry. 2003; 62: 929–37. 2. Theodoridis et al. Mass Spectrom Rev. 2011; 30: 884–906. 3. Bajad et al. Methods Mol Biol. 2011; 708: 213–28. 4. Xia et al. Nucl. Acids Res. 2012; 37: W652-660. 5. Gowda et al. 2014; 86 (14): 6931-6939. 6. Eric et al. Nat Rev Genet. 2010; 11(9): 647–657. A standalone software tool has been developed for supporting comprehensive metabolomic data analysis workflow.

POSTER 312

Disparate Metabolomics Data Reassembler: A Novel Algorithm for Agglomerating Incongruent LC-MS Metabolomics Datasets

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In the past several years, LC-MS metabolomics has risen to become a crucial platform for studying metabolic processes and small molecule characterization in biological systems. However, this burgeoning field still faces numerous fundamental shortcomings that can potentially hinder its development. As a whole the field is still very fractured, as the nature of the instrumentation, and of the information produced by the platform essentially produces “islands” of datasets that are often incomparable to one another. The non-interoperable nature of LC-MS data sets prevents the incremental aggregation of knowledge in the form of universal databases, which has been an important driver for accelerating the development other high-throughput “omics” platforms such as genomics. As such, we have developed a novel algorithm, called Disparate Metabolomics Data Reassembler (DIMEDR), which attempts to bridge the inconsistencies between incongruent LC-MS metabolomics datasets of the same biological sample type. Initially, a single “master” dataset is postprocessed via traditional means of peak identification, alignment, and grouping. DIMEDR utilizes this master dataset as a progenitor template by which data from all subsequent disparate datasets are reassembled and integrated into a unified framework that maximizes

spectral feature similarity across all samples. This is accomplished by a novel procedure for universal retention time correction and comparison via identification of ubiquitous features in the initial master dataset, which are subsequently utilized as internal standards during integration. For demonstration purposes, two human urine metabolomics datasets from previous studies were unified via DIMEDR, which enabled meaningful analysis across two otherwise incomparable datasets. The first study consisted of 280 urine samples collected from 95 patients undergoing total body irradiation (TBI), while the second study consisted of 40 urine samples collected from 40 colorectal cancer (CRC) patients. All data was acquired by Ultra-Performance Liquid Chromatography coupled to time-of-flight mass spectrometry, however these two datasets were acquired many months apart, making direct comparisons very difficult. Due to its size, DIMEDR utilized the TBI dataset as the progenitor template by which data from the CRC dataset would be reassembled and integrated into. After initial preprocessing of the positive mode TBI dataset, which yielded 11,119 unique spectral features, DIMEDR identified 195 ubiquitous features (present in more than 90% of TBI samples) for universal retention time correction and comparison. Reassembly and integration of the CRC data yielded an agglomerated dataset with 25.9% of features (with respect to the TBI feature count) shared by both TBI and CRC samples, and an additional 2,676 features unique to the CRC samples. DIMEDR easily outperformed standard methods of combining two datasets, which consist of using a simple parts per million (ppm) error threshold and maximum time difference for matching m/z values and retention times, respectively. Using a 15 ppm threshold and a 45s retention time difference, only 8.5% of features were matched between the two datasets, which is outperformed by DIMEDR by a factor of 3. DIMEDR is one of the first attempts at creating novel computational methodologies and solutions for unifying incongruent LC-MS metabolomics datasets.

POSTER 313

Comparative evaluation of open access software used in liquid chromatography-mass spectrometry based untargeted metabolomics

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LC-accurate mass MS/MS is widely used in metabolomic research, screening biological samples and comprehensively quantifying and identifying small molecules in order to gain insight into the physiological and chemical conditions of the cell. While targeted and multitargeted methods using MRM methods give very precise data, such methods are less suitable for discovery driven research. On the other hand, data processing for untargeted methods involve complex procedures yielding hundreds to thousands of peak findings that are hard to validate. In this study, methods were developed and optimized for MZmine 2.10, XCMS online, and MS-DIAL using plasma lipidomic data for testing the results of open access software solutions. Extraction of plasma lipids was carried out on replicate samples using a bi-phasic solvent system of cold methanol, methyl tert-butyl ether (MTBE), and water. After evaporation and re-suspension of an aliquot, extracts were analyzed using a Waters CSH C18 column with a 15-min gradient. Samples were analyzed with an Agilent 6530 QTOF-MS operated in ESI(+) to obtain different lipid species. 14 internal standards were added to the samples and were used as a benchmark to develop an efficient and reproducible data processing method capable of recovering all of the internal standards. The size and quality of the peak list was evaluated by investigating false/true positives and false/true negative findings. Methods were developed for MZmine 2.10, XCMS online, and MS-DIAL. The MZmine data processing method includes a mass detection of centroid data with a minimum height threshold of 2,000. The chromatogram builder feature was then used with a 0.1 minute minimum timespan requirement, a minimum height requirement of 2,000, and an m/z tolerance of 0.01 or 10ppm. Peaks were deconvoluted using Local Minimum Search, followed by the removal of isotopic peaks, and then alignment of the peak list data for all of the replicate samples into one master peak list. Any repeated peaks in the peak list that had an m/z of +/- 0.02 or a retention time of +/- 0.1 min of each other were removed from the list. Any peaks not detected were re-integrated or gaPoster filled if they were present in over 40% of the samples. This generated a peak list of more than 1,600 features; over 80% of these features had less than 20% relative standard deviation (RSD). The internal standards were among the

many identified features in the peak list and had RSDs less than 12%. The method designated for UPLC-QTOF on XCMS online was used to process the data and generated a peak list of over 2,000 features with all of the internal standards recovered. The default method for MS-DIAL was used and improved stePoster wise to yield a quality peak list of over 2,500 features; over 70% of these features had RSDs less than 20%. The internal standards were among the many identified features in the peak list and had RSDs less than 12%. An additional method for MS-DIAL was developed for data collected in MS/MS data-independent mode. The peak lists generated from MZmine 2.10, XCMS online, and MS-DIAL (MS1 and data independent MS/MS) were investigated for both abundant and low abundant peaks in retention time environments with many or with few co-eluting compounds. Compares the efficiency and reproducibility of data processing methods for untargeted lipidomics data using freely available software; including novel MS-DIAL.

POSTER 314

A New Automated Platform for Concurrent LC-MS and GC-MS Metabolomics

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Owing to the enormous metabolite diversity, sample preparation remains a crucial step of each method used for metabolomic analysis. Various microextraction procedures and, frequently, labeling of metabolites with natural and 13C-labeled signatures represent common approaches in mass spectrometry based metabolomics. We have developed a new workflow for metabolomics of protic metabolites in biological fluids and tissue extracts. It involves simultaneous metabolite labeling with common and 13C-labeled ethyl chloroformates combined with liquid liquid microextraction of the treated metabolites, sample extract transfer into separate vials, subsequent LC-HRMS and GC-MS analysis and automated data processing by an in-house developed Metabolite Cloud toolbox. The running workflow has been examined in the in-situ metabolomic analysis of cancer kidney cell extracts and urine. The unattended sample preparation was performed on an instrument-built or a stand-alone workstation developed by modification of an RTC autosampler corpus (Analytics, Zwingen, Switzerland). The in-house programmed operations comprise sample aliquot transfers, addition of internal standards, reagents, vortexing, dilution, mixing, withdrawal of an organic layer, exchange and cleaning of syringes followed by injection of the final sample extract into a UHPLC or GC chromatograph. The labeled metabolites are detected after LC and GC separation by any of the common MS ionizations; ESI, APCI and EI, PICI, APCI, respectively. The acquired data are moved into a remote Metabolite Cloud toolbox for non-targeted processing. Quantitative data are obtained by means of a vendor instrument quantitation software. From the tested metabolite set, more than 300 metabolites including amino acids, small peptides, nucleosides, biogenic amines, organic acids, steroids and other lipids were successfully subjected to concurrent LC-HRMS and GC-MS analysis by the developed platform. While LC-MS enabled analysis of nearly 200 metabolites, the GC-MS separation efficiency was capable to cover about 240 metabolites, primarily structurally closed amino acids and organic/fatty acids, of which about 90 analytes were amenable to MS analysis by both separation techniques. The tested metabolites provided well-defined labeled products with predictable ESI, APCI, EI and PICI mass spectra. Furthermore, complementary 13C-labeling, HRMS and MS/MS scanning facilitates metabolite identification by assignment a number of the labeled protic groups, their nature, the metabolite exact mass measurement and elemental composition. The developed approach has been proved useful for comparative metabolomic analysis of target sample and control sets. The platform has been evaluated by metabolite analysis in different biological matrices. Its performance in metabolite identification, quantitation and non-targeted metabolomics will be demonstrated on comparative analysis of normal and tumor kidney tissue extracts and urine samples. The Czech Science Foundation, project No. 13-18509-S (metabolomics),

the Czech Technology Agency, No. TA04011751 (technology). New automated metabolite analysis strategy for targeted and non-targeted MS based metabolomics has been developed.

POSTER 315

A rapid screening method to identify chemical components of a traditional herb using novel Informatics UNIFI Platform

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The efficacy of Traditional Chinese Medicine (TCM) has been affirmed by thousands of years of history and modern clinical practice. However, to reveal the material base and the mechanism of efficacy still remains to be one of the key challenges in today's research for TCM, especially to rapidly understand all of the chemical components from TCM. In this work, we adapted a rapid and effective strategy to holistically evaluate the quality of TCM samples using UPLC/Qtof MS coupled with a novel informatics platform. The example product used for this analysis was Ziziphi Spinosae Semen (ZSS). In this work, we adapted a variety of tools from the informatics platform for the purpose of carrying identifications for target and non-target purposes. Sample was provided by Hebei Yiling Pharma Group Co., Ltd and authenticated by Dr. Qing-Cun Tian. The powder (3.0 g) was suspended in 25 mL 60% MeOH/H2O, ultrasonically extracted for 60 min at room temperature and filtered through a 0.22 μm nylon filter film prior to injection. The chromatographic separation was carried out on an UPLC BEH C18 column (2.1mm × 150 mm, 1.7 μm) at temperature of 35°C. Mobile phase consisted eluent A (0.1% formic acid in water) and B (0.1% formic acid in acetonitrile) with a flow rate of 0.4 mL/min. Both ESI+ and ESI- experiments were performed over m/z 50 to 1500. Data mining as well as report generation were carried on with vendor provided informatics platform. The extracted herbal sample was analyzed by UPLC and high resolution QTof MS including both low and high energy (fragment ions) data from a single injection. The data processing and analysis were conducted using a novel informatics platform provided by the instrument vendor, which offered a streamlined workflow that incorporates a Traditional Medicine Library. This resulted in a simple and efficient process for the identification of the chemical ingredients from the traditional herb. In addition, an in house library containing entire list of ALL components from ZSS reported in the literature was created to compliment the commercially available Traditional Medicine Library. Utilizing the Natural Products Application workflow, and adapting both The Traditional Medicine Library as well as the in-house ZSS library, all compounds already reported from literatures were easily identified (69 identified). All components not listed from either libraries were then listed as unmatched peaks in the result browser. Further identification was carried on by utilizing tools provided from the informatics platform such as: structural elucidation tools, common fragment search, Mass Defect Filter etc.. This exercise lead to the discovery of 44 additional compounds, all were identified from this TCM sample for the first time. As a result, for ZSS sample, from single LC/MS injection, total of 113 compounds were identified, among which 44 were novel to this sample. A novel strategy to profile known and unknown chemical components in traditional medicine from a single injection.

POSTER 316

A Peptide-Based Method for 13C Metabolic Flux Analysis in Microbial Communities

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Microbial communities underlie a variety of important biochemical processes ranging from underground cave formation to gold mining or the onset of obesity. Metabolic fluxes describe how carbon and energy flow through the microbial community and therefore provide insights that are rarely captured by other techniques, such as metatranscriptomics or metaproteomics. The most authoritative method to measure fluxes for pure cultures (13C Metabolic Flux Analysis, 13C MFA) consists of feeding the cells a labeled carbon source and deriving the fluxes from the ensuing metabolite labeling pattern (typically amino acids). Since we cannot easily separate cells of metabolite for each species in a community, this approach is not generally applicable to microbial communities. Here we propose a new type of 13C MFA that infers fluxes based on peptide labeling, instead of amino acid labeling. The advantage of this method resides in the fact that the peptide sequence can be used to identify the microbial species it originates from and, simultaneously, the peptide labeling can be used to infer intracellular metabolic fluxes. Peptide identity and labeling patterns can be obtained in a high-throughput manner from modern proteomics techniques We show that, using this method, it is theoretically possible to recover intracellular metabolic fluxes in the same way as through the standard amino acid based 13C MFA, and quantify the amount of information lost as a consequence of using peptides instead of amino acids. We show that by using a relatively small number of peptides we can counter this information loss. We computationally tested this method with a well-characterized simple microbial community consisting of two species. We introduce a method that, for the first time, can measure fluxes for microbial communities using 13C labeling experiments.

POSTER 317

Global Metabolomic Analysis of 13C-enriched Mouse Tissues using 13C NMR

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1H NMR is the most commonly used NMR method in metabolomics. The 13C nucleus has a low natural abundance resulting in lower sensitivity than 1H. Isotopic labeling of mixtures can greatly increase the S/N of 13C spectra; however, large homonuclear couplings complicate their interpretation. One way to take advantage of these couplings is through the use of 2D NMR, more specifically, 2D 13C-13C INADEQUATE. 2D INADEQUATE uses 13C-13C homonuclear coupling allowing for the direct assessment of carbon-carbon backbone. Our goal is to successfully extend both 1D and 2D 13C NMR to complex organisms such as mice with the aim of understanding the distribution of metabolites across mouse tissues. Three mice were fed a custom diet partly consisting of 13C isotopically labeled hydrosylate (amino acids) for 14 days upon reaching adulthood. 13C-enriched mouse tissues were collected and metabolites extracted. Dried extract was resuspended in 1:4 D2O: MeOD and placed in 1.5 mm NMR tubes. All samples were analyzed using the 1.5 mm HTS cold probe (Ramaswamy 2013) at the U Florida. From the 13C-labeled livers with sufficient metabolite concentrations we were able to collect 2D 13C-13C INADEQUATE spectra. Chemical shifts of metabolites characterized using INADEQUATE were identified in the 1D 13C spectra of tissues whose mass was not sufficient for INADEQUATE data collection. Our lab has developed a simple and automated approach called INETA to perform untargeted analysis of INAEQUATE datasets. We collected 2D 13C-13C INADEQUATE on mouse livers and identified metabolites using INETA. Approximately 30 metabolites were successfully annotated in the INADEQUATE and 75 networks were found. The lower weight and overall metabolite concentrations prevented INADEQUATE spectra to be collected from other tissues. We adjusted our approach by using INETA to identify metabolites and corresponding 13C chemical shifts in the mouse liver INADEQUATE. 13C chemical shift information was then used to annotate the 1D 13C spectra of all tissues. The relative concentrations of these metabolites were compared to determine the differences in metabolic profiles between the various tissue types. We observed differences in essential and non-essential amino acids of the labeled diet, which may be caused by the accumulation, or depletion of free essential amino acids due to high or low protein turnover in the various tissues. Sugars such as glucose are predicted to have higher levels in the liver and the plasma compared to other tissues. Due to its need for a constant supply, the brain has little to no detectable glucose. Changes in the levels of these metabolites may lend insight

into the differing metabolism between the various tissue types. Our analysis demonstrates the ability to perform 13C global (untargeted) metabolomics on isotopically labeled mammalian tissue using 1D and 2D 13C NMR. Qualitatively analyzing the metabolic profiles of tissues helps us to understand the compartmentalization and importance of metabolites in animal tissues.

POSTER 318

isoMETLIN: A Database for Isotope-Based Metabolomics

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We recently introduced a suite of bioinformatic resources for comprehensive tracking of isotopic fates unbiasedly in the metabolome, an experimental design we refer to as “isotope-based metabolomics”. The basis of our bioinformatic approach for interpreting these results is a new database, isoMETLIN. isoMETLIN is an extension of METLIN that has been developed specifically to identify metabolites containing isotopic labels. isoMETLIN enables users to search all computed isotopologues derived from METLIN on the basis of mass-to-charge values and specified isotopes of interest, such as 13C or 15N. Additionally, isoMETLIN contains experimental MS/MS data on hundreds of isotopomers. The MS/MS spectra of additional isotopomers have been computationally generated, included, and in some cases validated. Isotope standards were diluted to approximately 1μg/mL in water, methanol, or a combination of each depending on the compound. Each standard was analyzed by using a HPLC system interfaced to an ESI-QTOF and Quadrupole-Orbitrap (Agilent Technologies 1200 series, 6520 QTOF, and ThermoFisher Scientific Q Exactive Plus). The standards were analyzed by flow injection using an isocratic 50% water and 50% acetonitrile mobile-phase composition. A narrow window (1.3 Da) was used to acquire MS/MS data for each standard at 4 different collision energies. Raw data were processed by using an in-house R script for extracting and normalizing each MS/MS spectrum. Fragments with relative intensities less than 0.5% of the most intense fragment were excluded. isoMETLIN, which is an analog of the widely used METLIN database, is designed to facilitate the identification of both isotopologues and isotopomers. This resource is meant to power studies in which the metabolites that are isotopically enriched are unknown (i.e., untargeted tracking of isotopic fates). While identifying isotopologues can be biochemically informative, differentiating isotopomers generally provides greater insight into metabolic pathways. Currently, isoMETLIN includes millions of isotopologues as well as experimental MS/MS data on hundreds of isotopomers. At this time, isoMETLIN supports searches of 13C, 15N, 2H, 18O, or any combination of these atoms. isoMETLIN was populated by calculating the masses of all 13C, 15N, 2H, and 18O isotopologues for each compound in METLIN. It is important to note that isoMETLIN is particularly well suited for investigators that are trying to identify features that may or may not be isotopically enriched. For example, this is common in clinical environments when patients are fed stable isotopes and no unlabeled control is available. To enhance the power of isoMETLIN for interpretation of such biological samples, we have introduced a “mixing function” that allows users to average the MS/MS data from pure isotopomers to generate a mixed MS/MS spectrum to be matched against the MS/MS spectrum of a sample. isoMETLIN is the first database for the isotope-based metabolomics, which enables researchers to track the transformation of labeled metabolites unbiasedly.

POSTER 319

MetExtract II: A novel and powerful data processing software for stable isotopic labelling assisted and LC-HRMS(/MS) based untargeted metabolomics

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Stable isotopic labelling (SIL) assisted approaches gained much popularity in recent years and are being increasingly used in metabolomics applications. However, currently only few software tools make use of SIL derived isotope clusters for efficient data processing. In this respect, we present MetExtract II, a novel software package for SIL assisted and LC-HRMS based untargeted metabolomics. It consists of three modules (A-C) which support A) the global detection of all metabolites of a biological system, B) the specific detection of only biotransformation products of a labelled tracer under investigation and C) the SIL assisted annotation of LC-HRMS/MS spectra. All modules have been designed to make use of 13C-labelling experiments, work with different LC-HRMS instruments and support fast polarity switching ionisation. MetExtract II detects metabolites first in the m/z dimension using unique isotope patterns originating from the simultaneous LC-HRMS analysis of native and highly isotope enriched samples or tracer substances. Subsequently, MetExtract II also utilises the chromatographic information and tests if the native and labelled metabolites show perfect co-elution. All metabolite ions passing these verification criteria are subject to several post-processing steps that annotate the ions with putative heteroatoms, automatically convolute different ions from the same metabolite and perform internal standardisation for every metabolite. Finally, the results of different LC-HRMS runs are combined using their m/z values, retention times and number of labelled atoms into a comprehensive data matrix. MetExtract II processes full scan LC-HRMS(/MS) data for the comprehensive detection of all metabolites present as native and uniformly or partially 13C-labelled metabolite ions. In untargeted metabolomics applications (module A), it enables the comprehensive detection of all metabolites of a biological system under investigation and each detected metabolite is annotated with its exact number of carbon atoms. Moreover, with an appropriate sample preparation protocol, MetExtract II automatically performs internal standardisation, which improves relative quantification and accounts for putative matrix effects. Module B of the MetExtract II package allows searching specifically for biotransformation products of a certain native and 13C-labelled precursor-substance under investigation. Thus, only such metabolites that are either direct or indirect descendants of the tracer are reported. This allows specifically studying metabolic pathways which affect the studied tracer and also enables the unbiased discovery of novel biotransformation products of the tracer. Finally, module C facilitates further characterisation of unknown metabolites by annotating LC-HRMS/MS fragment and precursor ions originating from native and labelled precursor ions. MetExtract II is presented with wheat samples either treated with the pathogenic fungus Fusarium graminearum or water: All LC-HRMS accessible metabolites extracted with module A give a clear separation into the two conditions, which gets more pronounced after 32 hours of treatment. Putative metabolite annotation showed that about 20% of the extracted metabolites could be matched to known wheat substances. Subsequently, phenylalanine-derived metabolites, many of which are known to be involved in defence in planta, were studied using module B. The majority of all phenylalanine-containing metabolites showed either decreased or increased abundance between the Fusarium- and mock-treated samples. Metabolites significantly differing between the experimental conditions were further characterised with module C of the presented software. The MetExtract II package, associated documentation and sample data are available free of charge for academic use. Software for SIL assisted untargeted metabolomics; enables global metabolome annotation, tracer-fate studies and LC-HRMS/MS fragment annotation

POSTER 320

Increasing signal to noise ratio in metabolomics studies

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Metabolomics is applied in many different areas of biology in order to understand metabolic responses/mechanisms associated with various perturbations, e.g. genetic modifications, response to toxins as well as development and treatment of disease. Depending on the nature of the perturbation the magnitude of the metabolic response can differ enormously. A common setup in metabolomics is to have two pre-defined sample groups, e.g one case group that has been subject to the perturbation and one control group. The data is evaluated by some statistical method to find metabolites or metabolite patterns

that differs significantly. A change is considered significant if the response (the signal) is “larger” than other systematic variation (the noise) caused by age, gender and diet etc. To enable interpretation/understanding of weak metabolomics responses the “signal to noise ratio” in metabolomics studies has to be increased. One way of doing so is to decrease the noise. This can be achieved by using dependent statistical analysis to focus on the effect of the perturbation over all matched or dependent samples. However, in order use a dependent analysis strategy the study design must allow it. This is sometime obvious when the same sample or subject is analyzed before and after a perturbation but could in theory also be achieved by matching samples from the two groups (case and control) by relevant factors such as age, gender and storage time etc. We describe how strategies for sample matching and design of sample runorder can be of help in reaching the goal of an increased signal to noise ratio in metabolomics data. In addition we are presenting and apply a novel method allowing multivariate analysis of dependent samples named OPLS-EP. We show that the combination of a sophisticated study design, i.e. sample matching and runorder design, and multivariate analysis focusing on the effect of the perturbation by OPLS-EP do increase the signal to noise ratio in metabolomics studies. A novel runorder design and a novel method for multivariate analysis of dependent samples will be presented.

POSTER 321

3D time-dependent fluxomics: probing metabolic flux across the circadian cycle using computational and experimental approaches

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Recent studies suggest that not only what we eat, but when we eat, play a role in the development of metabolic disorders. Indeed, the detriments of a high fat diet can be largely ameliorated by time-restricted feeding. Systems-level research on circadian rhythms has focused on finding oscillatory patterns in ‘omics data in both altered feeding paradigms and genetic clock disruption. While this data can provide snapshots of metabolism, the inherently dynamic nature of these rhythms cannot be proven with static measurements. We hypothesize mechanistic explanations of metabolic abnormalities in disrupted rhythms and altered feeding paradigms can be found by delineating metabolic flux. Here we incorporate computational and analytical tools to assess flux in major metabolic pathways across the circadian cycle. We employ constraint-based modeling (CBM) to generate untargeted predictions of metabolic flux across the circadian cycle using publicly available circadian expression datasets. Our objective is to compare these predictions of major metabolic pathways and metabolite transport processes across multiple metabolic tissues in different feeding paradigms and in the presence of a dysfunctional clock. Additionally, these predictions of diurnal metabolic changes can guide experimental flux experiments. We have also recently developed multiple metabolomics methods using liquid chromatography mass spectrometry (LC-MS) to analyze polar metabolites. With these tools, coupled with recent advancements in untargeted metabolomics data processing, we can utilize differential labeling analysis and metabolic set enrichment analysis to identify pathways which utilize isotopically labeled nutrients in vitro. We initially performed CBM-based flux predictions using circadian microarray data in the mouse liver. We noted large changes in major metabolic pathways, including central energy, amino acid, and fatty acid metabolism, among others. We plan to verify these predictions by experimentally testing glycolytic flux in MMH-D3 hepatocytes, a recently validated in vitro model of circadian rhythms. We will be using targeted and untargeted metabolomics methods on triple quadrupole and quadrupole time-of-flight mass spectrometers, respectively, for these experiments. Given the complexity of co-optimizing LC-MS analyses on metabolites with such a diverse set of physiochemical properties, we employed a design of experiments approach to optimize both the targeted and untargeted methods. We now have assembled the necessary tools, including in silico computational approaches, and both targeted and untargeted LC-MS of polar metabolites, to begin to gauge the dynamics of metabolism across the circadian cycle. Metabolic flux predictions and analysis across the circadian cycle to assess time of feeding vis-à-vis the genetic circadian clock.

POSTER 322

Development of an integrated workflow for high-throughput 13C-Metabolic Flux Analysis

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Despite increasing needs in systems biology, synthetic biology, biotechnology, and human health, the application of fluxomics is still limited. Fluxomics is originally a low throughput method, which limits the number of organisms, strains and conditions which can be investigated. To increase fluxomics throughput while reducing experimental costs and human efforts, a complete workflow for automated and parallelized 13C-fluxomics experiments was set-up. The workflow includes both new experimental and computational tools, as well as an original robotic platform for fully automatized 13C-labelling experiments The strategy was to optimize the complete fluxomics workflow, including i) the optimal design of isotope labeling experiments, ii) the automated and parallelized cultivations of organisms with 13C-labelled substrates and labelled sample collection using an original robotic platform, iii) optimizing the analytical methods for measuring isotopic data using NMR or MS, or both, with reduced analytical times, iv) the processing and interpretation of isotopic data. In addition, a strategy based on 13C labeled standard was produced to evaluate potential analytical biases and to qualify the analytical platforms for isotopic measurements. Finally, a set of bioinformatics and mathematical tools were developed to optimize and speed up data processing and flux calculations Optimal design of 13C-labelling experiments is a key step in improving the number of fluxes that can be calculated from a set of isotopic data and precision of flux values. We developed IsoDesign (Millard P & al – Biotech. Bioeng 2014) software that runs on top of influx_s (Sokol & al. Bioinformatics 2012) and optimizes the label input to maximize these parameters. A novel platform was developed for automated HT-fluxome profiling of metabolic variants (Heux.S & al – Metabolic Eng. 2014). This robotic system prepares, runs, monitors and controls 48 micro-scale fermentations in parallel (pH, temperature, stirrer speed). The robotic platform performs fully automated harvesting of cells and extraction of metabolites for downstream analysis (Millard.P & al – Anal. Biochem 2014). Fast and reliable methods for measuring the isotopic content of metabolites were established for NMR (Cahoreau.E & al – Anal. Biochem 2012, Giraudeau.P & al – Chemphyschem 2012) and LC-MS/MS. Automated Enrichment Data Extraction (AEDE) was developed to extract meaningful information from 1D-NMR spectra, while IsoCor (Millard.P & al – Bioinformatic 2011) is used for MS(/MS) data. Because analytical measurements are prone to various biases, we developed a complete theoretical and experimental framework for the qualification of analytical platforms from biologically-produced metabolites with fully controlled and predictable labeling patterns (Millard.P & al – Anal.Chem 2014). The integrated fluxome platform was applied to a set of Escherichia coli mutants with varying levels of a single enzyme and grown on two different 13C-labelled carbon sources (Heux.S & al – Metabolic Eng. 2014). We were able to generate 20 fluxome profiles per day, which, as far as we know, is a major advance in the field. This approach used here to deduce metabolic flux in E. coli, will also be used to resolve fluxes in mammalian cells in a high throughput manner This work significantly expands the application and throughput of 13C-Fluxomics while increasing the reliability of the biological insights

POSTER 323

DynaMet, a fully automated pipeline for dynamic labeling LC-MS data

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Time-course labeling data provide crucial information about the dynamics of metabolites in biological systems because the speed of labeling incorporation into metabolite pools depends on the corresponding pathway activities. Isotope labeling experiments have a long tradition in the elucidation and fundamental understanding of essential pathways such as the tricarboxylic acid cycle1 and the glyoxylate cycle2. However, LC-MS analysis of cell extracts from dynamic labeling experiments are highly complex since isotopic patterns of metabolite ions change significantly overtime and grouping of those patterns from different samples is highly challenging. Herein, we introduce DynaMet a comprehensive, fully automated data analysis pipeline for detection and profiling of labeled metabolites starting from LC-HRMS raw data

sets generated from dynamic labeling experiments. LC-MS data originated from previous study of B. methanolicus MGA 3, a recently described thermophilic strain of biotechnological relevance capable to grow on methanol as sole carbon source3. Samples of a time course labeling experiments using 13C methanol, which were taken at various time points after carbon source switch. All samples were analyzed with an LTQ-Orbitrap instrument operating at unit resolution of 60'000 (at m/z 400) applying nanoscale ion-pair reversed-phase HPLC-MS method4. After conversion of raw data files into mzXML format data were analyzed independently with DynaMet. In addition, labeling profiles of selected core metabolite ions were extracted from data by a targeted approach and automatically generated parameters of kinetic fits were determined independently using Prism software. DynaMet enables automated, untargeted extraction of metabolite labeling profiles by grouping features in different samples with isotopic patterns changing over time. This was achieved by combining previously introduced peak finding algorithm with developed peak grouping algorithm called isotope_regrouper'. Isotope_regrouper is fast and allows distinguishing and annotating isotope shifts from different elements and is able to group peaks to features with arbitrary intensity profiles from high mass resolution data. Moreover, integrated tools for expressive data visualization such as labeling profile plots or feature grouping flat clustering analysis based on kinetic fit parameters enhance result inspection. To validate DynaMet we used data from time course labeling experiments with Bacillus methanolicus MGA3. As reference, we manually determined labeling profiles for a set of metabolites involved in core metabolism. The analysis tool found all selected metabolites and labeling profiles were highly similar to those obtained by manual analysis. In total, DynaMet extracted 282 features showing dynamic labeling within ten minutes whereof 249 could be fitted by implemented kinetic models. Feature identification against an integrated KEGG database resulted in 134 matches corresponding to 95 different metabolites, which cover multiple reaction pathways of core one carbon metabolism and major biosynthetic routes. Moreover, provided filter functions for data inspection allowed straight forward extraction of potential carrier of one-carbon molecules (features with labeling profiles showing fast incorporation of one carbon atom) and dominating carrier could be identified as formyl-tri- and tetra-glutamyl-tetrahydrofolate and formyl-tetraglutamyl-tetrahydrofolate showing its high potential for elucidation of new metabolic routes. Manual inspection of extracted labeling pattern revealed around 7% false positives for all features, and <1% false discovery rate for automatically extracted and fitted features. DynaMet will be made freely available as an extension package for Python based eMZed 2, an open source framework built for rapid development of LC-MS data analysis workflows with Python. DynaMet's integrated data processing modules greatly facilitated the analysis of dynamic labeling experiments and drastically accelerates metabolic pathway discovery.

POSTER 324

Automated pipeline for quality control of large scale LC-MS discovery data in metabolomics using MZmine

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Quality control (QC) is a crucial part of large-scale LC-MS experiments, which is used to verify that the sample preparation, LC and MS methods meets the required accuracy and precision (including repeatability and reproducibility). QC should identify potential problems as early as possible in order to counteract them and save valuable samples and analysis time. Manual QC is too time demanding, error prone and cannot be provided in real-time. Therefore, the effective automatisation of the QC process is essential. Here we present a QC pipeline, which is being implemented in the newly established clinical research environment. Before and after sample extraction, a set of internal standard are added to monitor the extraction and LC-MS methods. External standards (standard mixtures, blanks and serum samples) are run throughout the sample batches. An automated QC pipeline system is set up in order to analyse these standards with the following four automated steps: i) Transfer of the acquired raw data to a storage server. ii) Conversion of raw data files to mzML files using ProteoWizard (D. Kessner, et al., 2008). iii) Targeted peak detection of standard compounds and export of results to a database using MZmine 2 (T. Pluskal, et al., 2010) and iv) Data analysis and visualisation of peak information and identification of fluctuations outside a certain threshold. Three specific software tools were developed as part of the QC pipeline. The first runs on the MS acquisition computers and transfers raw data files to a storage server after the acquisition of the run has finished. The second software runs on a processing server and

converts the raw data files to the open mzML format. After conversion, the mzML files are processed by the software using the batch mode of MZmine to perform a targeted peak detection for the standard compounds. The final step in the batch exports all the peak data to a database. Finally, a web application was developed which visually presents the data in QC plots and allows the user to set threshold values and see which samples are not within the accepted threshold for a given parameter (e.g. m/z, RT, peak area, peak height, duration, carryover, #data points, FWHM, tailing factor and asymmetry factor). Within minutes after the experimental run has finished the data is available in the web application, thereby providing a real-time feedback for the instrument operator on how the extraction and LC-MS methods are performing. Since all the QC data from the samples is stored in one database, it is possible to easily perform trend analysis on samples analysed over long periods of time. Data are normalised using the internal standards to address these changes between individual runs. And by integrating data from room sensors (temperature, humidity and CO2), it is also possible to correlate trends to surrounding room factors and thus be alerted if any deviations from normal conditions occur. Other than quality control, the external standards are also used to generate calibration lines for quantification purposes. Combination of multiple tools and software to implement an automated QC pipeline for LC-MS experiments.

POSTER 325

Compression and rapid visualisation of quantitative mass spectrometry data based on raw signal decomposition

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As archival of profile mode datasets becomes a requirement, there is a danger that the ever increasing size and scale of LC-MS metabolomics data becomes more opaque and inaccessible to practitioners. It is therefore critical that efficient compression and visualisation tools are available to facilitate archival, quality control, verification, validation, interpretation and sharing of raw MS data and the results of MS analyses. Currently MS data is stored as contiguous spectra. Recall of individual spectra is quick but panoramas, zooming and panning across whole datasets necessitates processing/memory overheads impractical for interactive use. Moreover, visualisation is challenging if significant quantification data is missing due to data-dependent acquisition of MSn spectra or data independent acquisition approaches such as SWATH and MSE. In order to tackle these issues, we leverage our seaMass technique [Liao et al., IEEE ISBI 2014, http://dx.doi.org/10.1109/ISBI.2014.6868123] for raw signal decomposition. LC-MS data is modelled as a two-dimensional surface through selection of a sparse set of weighted B-spline basis functions from an over-complete dictionary. By ordering and spatially-partitioning the weights with an R-tree data model, efficient streaming visualisations are achieved. In a recent publication [Zhang et al., Proteomics 2015, http://dx.doi.org/10.1002/pmic.201400428], we describe our core MS1 visualisation software for proteomics. Here, we present the framework for metabolomics, including new tools for SWATH/MSE and to handle centroided data, and an approach packaging mzML and seaMass decompositions within a HDF5 container for realising a highly compressed format with rapid 2D visualisation capability. First we assessed how close reconstructed seaMass decompositions are to the original raw data. seaMass is dependent on the desired compression ratio set through parameter λ. With λ = 0.06, ToF data is compressed to ~5% original size, with mean absolute error per data-point ~1.5 ion counts. Further investigation showed that percentage error is inflated for data points with an ion count less than 10, and reduces as peak intensity increases. The effect is a conservative data denoising, as the decomposition is not capturing non-smooth ion counting noise. By additionally storing the difference delta between the reconstruction and original dataset, lossless archival can be achieved. 2D data visualisations usually start with an overview of the whole dataset before zooming/panning to areas of interest. The effect of the missing data imputation is to minimise changes in curvature where data is absent. With 25,000 coefficients streamed out for an Orbitrap dataset overview, most of the peaks are revealed; only some subtle changes remain. Since there is dramatically more background information present in ToF data, around 2.5% of the coefficients are needed before there is no noticeable improvement, but this is still only equivalent to 0.125% of the original raw dataset size. R-tree decoding from disk varied from

118,048 to 530,214 coefficients per second, with image reconstruction at 800x600 performing similarly. At these rates, visually complete overviews take seconds to appear, and zoomed regions much quicker. Our computationally intensive but one-time decomposition step is key to subsequent interactive visualisation with little CPU or memory (<200Mb) overhead. This enables the possibility of implementing novel visualisation schemes integrating results and raw data across complete experiments, which would greatly facilitate QC, verification, validation and expert interpretation of MS analyses, beyond that of what we demonstrate here. The open-source software is available from http://seamass.net/viz/. Compression of quantitative LC-MS data with novel raw signal decomposition methodology and its streaming from disk for rapid 2D visualisation.

POSTER 326

RAMclust/RAMsearch: efficient post-XCMS feature clustering and annotation of MS-based metabolomics datasets.

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Chromatographically coupled mass spectrometry is a powerful tool for profiling, semi-quantitatively or quantitatively, a breadth of small molecules with sensitivity and selectivity. The complexity of these datasets has driven the development of informatics approaches for feature finding, retention time alignment, feature grouping, and annotation. However, the complexity of signals derived from a single compound is generally underestimated, resulting in poor spectral reproducibility, misannotation, and misinterpretation of individual mass signals. This limitation has driven us to develop informatics tools to improve the quality of post-XCMS data processing. RAMclustR is developed in R and is freely available. It is designed with memory constraints in mind, and operates on the scale of minutes, but can take an hour when peak shape similarity scoring is also used. The output is initially an R object containing a dataset of reduced dimensionality as compared to the input XCMS set, as well as spectra which are written to .msp format. These spectra can include MSE (indiscriminant MS/MS) spectra when available. This msp format is taken as input for RAMseach, a .NET-based GUI for performing batch spectral searching against NIST formatted spectral libraries. The results can be output in a format which can be reimported back into the ramclustR. RAMclustR feature similarity scores are calculated for all feature pairs in the input XCMS R object, where feature similarity is the product of individual similarities in correlation in intensity across the dataset, feature retention time, and peak shape. The contribution of each score is tunable using sigmoid functions, enabling the evaluation of results and adjustment, when necessary. The output datasets demonstrate improved injection reproducibility as compared to individual features, reduce false discovery error rate burden, and improve annotation quality. Annotation efficiency is dramatically improved by utilizing the output spectra from RAMclustR as input for spectral searching using RAMsearch, a novel GUI for batch searching and manual validation of search results. The output from RAMsearch is imported into RAMclustR, enabling the storing, visualization, and sharing of the evidence for a given annotation. These output are suitable as supplementary material upon publication of the dataset, to ensure transparency in the annotation process. This workflow reduces annotation time several fold by automating routine manual tasks. Further, it is designed to streamline the efforts that go into reporting annotation confidence, which will enable more robust, transparent, and accessible reporting of metabolomics data RAMclustR and RAMsearch collectively improve reproducibility, reduce user burden, improve statistical power, streamline annotation, and improve transparency.

POSTER 327

IPO: A Tool for automated XCMS Parameter Optimization

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The workflow of untargeted metabolomic data processing includes peak detection, retention time alignment and grouping. These tasks are already handled by tools such as XCMS. Its data processing can be adapted by various parameters to any kind of LC-MS data. Experienced users usually have settings at hand that produce quiet reliable results. But day to day differences in the experimental setup may still require customization of these settings. Customizing the settings can be challenging in terms of expertise and manpower and the customization results are not easily evaluated. A tool for automatic optimization would save time and provide settings for unexperienced users. The programming language R was chosen to ensure compatibility with XCMS. A design of experiments (DoE) approach was used to efficiently test different parameter levels using the R-package rsm. The results of these parameter levels were assessed by applying newly developed scores. These scores were evaluated using response surface models and the best scoring parameter levels were used in the following DoE as long as the respective scores increased. For evaluation of the optimization approach 132 serum samples were processed and pooled. LCMS analysis was done by a Thermo QExactive mass spectrometer and chromatographic separation was achieved using hydrophilic interaction chromatography. For both, the training and the test set, data from 15 different injections of the pooled sample were used. We implemented IPO, an R-package for automated optimization of XCMS parameters. Peak picking parameters are optimized separately whereas parameters for retention time correction and grouping are simultaneously optimized. For optimization of the peak picking parameters natural, stable 13C isotope peaks are identified. These isotope peaks are then used for calculation of a peak picking score (PPS). Thereafter, IPO minimizes relative intra-group retention time deviations within the optimization process of the retention time correction parameters. At the same time grouping parameters are optimized which is based on the classification of good and bad groups. The parameter settings acquired from the training set by IPO as well as the default XCMS settings were used on the test set. The reliable peaks increased from 1,406 to 4,552 and the total number of peaks decreased from 59,326 to 36,808 when we compared the data generated with default settings to the data generated with optimized settings. Also, the number of good groups increased from 706 to 1,046 whereas the number of bad groups decreased from 848 to 604. The average retention time deviations within the groups were reduced by 26% in the test set. We implemented an R-package to automatically optimize parameters of XCMS methods to increase the reliability of untargeted metabolomic data processing.

POSTER 328

Comparison of the Performance of Feature Selection Methods for Biomarker Discovery

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Metabolomics is an active area of research due to the potential it holds for disease diagnosis and management. Most studies are aimed at the identification of biomarkers for disease diagnosis. Several feature selection/data mining algorithms are available for data processing. We have developed a model quality assessment parameter for classification models termed Cluster Resolution (CR), which is used to guide an automated feature selection (biomarker discovery) algorithm using a hybrid backward elimination / forward selection algorithm. We compare the performance of our CR-guided approach with other feature selection algorithms. Algorithms are evaluated based on required computation time; model quality i.e. specificity, sensitivity and accuracy; robustness of the FS process and the chemical significance of the features that survive the process. SPME-GCxGC-TOFMS was performed on volatile compounds from smelly T-shirts. Data were analyzed in ChromaTOF and the peak tables imported into MATLAB. Evolutionary algorithms such as GA-SVM, GA-RF, GA-LDA and GA-QDA, CPOSTER ANN and CR-FS were developed in Matlab. MetaboAnalyst, a web based biomarker discovery toolkit was used as is. Input parameters for each algorithm were optimized. The data were permuted into 100 combinations of training and validation sets prior to passing the data to each discovery tool. This ensured that artefacts due to data ordering influenced each algorithm equally. Feature selection was performed using each algorithm. PLSDA and PCA models were developed using the selected features and the quality of the final predictive model was evaluated. GA-based algorithms require several inputs such as chromosome

length, initial population, mutation rate, selection rate and cross over rate. RF, CPANN and CR-FS require the optimization of number of trees; number of network nodes and epoch; and the backward elimination start number, respectively. The method optimization time is commensurate with the number of parameters to be optimized, and expands geometrically with the number of parameters. Hence GA-SVM, GA-RF, GA-LDA and GA-QDA require significantly longer to optimize. PCA model quality demonstrated by the confidence limit at which there is no overlap of an confidence ellipse around each class followed this trend, CR-FS > GA-RF>GA-SVM>GA-CPANN. All other algorithms showed no observable class separation. Model classification in PLSDA space showed a trend of: CR-FS > GA-RF>GA-SVM> MetaboAnalyst > GA-KNN > GA-LDA > GA-CPANN > GA-QDA. In terms of model optimization time, the CR-FS approach required 85 min (for the 100 permutations of input data) to yield an accuracy of 0.975. The next-fastest was RF, which required 107 min but yielded a model that had an accuracy of 0.958. The remaining algorithms required between 360 and 950 minutes to complete and yielded models with accuracies ranging from 0.744 to 0.942. It should be noted that these computation times are for the algorithm with already-optimized parameters and do not include the time required to optimize the individual algorithm. Overall the CR-FS approach was shown to deliver better models in less time with fewer parameters needing optimization. Cluster Resolution Guided Biomarker Discovery: An approach that converges quickly to a more stable, robust, and reproducible solution.

POSTER 329

Automated Software for Quantitative Metabolomics

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Metabolomic assays are normally very manually intensive exercises requiring considerable skill, time and patience. They also often need custom software to facilitate identification and/or quantification of metabolites. These requirements can place severe limitations the throughput and reproducibility of many metabolomics experiments. In an effort to overcome these barriers, The Metabolomics Innovation Centre (TMIC) has developed a number of software tools to facilitate automated or semi-automated compound identification and quantification on common NMR, GC-MS and LC-MS platforms. Manual NMR, GC-MS and LC-MS identification methods were carefully assessed and processes that were best-suited for automation were identified. Reference compounds, spectra and spectral libraries for NMR, GC-MS and high resolution LC-MS were compiled and used for testing, refining and further development. Fully automated software was developed for spectral profiling and compound identification/quantification for NMR (Bayesil), GC-MS (GC-AutoFit) and LC-MS (CFM-ID and MyCompoundID). Bayesil (http://www.bayesil.ca) is a fully automated system that performs both 1D NMR spectral processing and profiling over 60 different metabolites in CSF and serum and over 100 different metabolites in urine with >90% accuracy. Bayesil is capable of processing spectra in 2-3 minutes compared to 20-40 minutes (for a human expert) or 5-6 hours (for the next best automated software package). GC-AutoFit (http://gcms.wishartlab.com) supports automatic GC-MS spectral alignment, peak picking, retention index and peak area calculation, compound identification and quantification from standard GC-MS run. It is capable of identifying >40 different metabolites in serum and >70 metabolites in urine with 95% average accuracy. The process time is <2 minutes per sample on 2.4 GHz Core2 Duo Laptop. CFM-ID (http://cfmid.wishartlab.com/) provides a method for accurately and efficiently identifying metabolites in spectra generated by ESI-MS/MS. The program uses Competitive Fragmentation Modeling to produce a probabilistic generative model for the MS/MS fragmentation process and machine learning techniques to

adapt the model parameters from data. Predicted MS/MS spectra have been generated and annotated for more than 50,000 compounds from the HMDB and KEGG. MyCompoundID (http://www.mycompoundid.org/) is a web-based resource for identification of compounds of interest based on chemical properties of a molecule, such as accurate mass and fragment ion spectral pattern generated by mass spectrometry (MS). Based on extensive testing with defined mixtures and real biological samples, these automated software systems consistently perform with sensitivity and specificity greater than 90% for compound identification and/or quantification. These software programs and web servers represent some of the first freely available tools for automating metabolite identification and quantification.

POSTER 330

xMSannotator: an R package for network-based annotation of high-resolution metabolomics data using biological, chemical, and environmental databases

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Numerous efforts have been undertaken over the last decade to improve the analytical technologies and data extraction algorithms for high-resolution mass spectrometry. These efforts have enhanced the coverage of chemical detection for metabolic profiling and environmental bio-monitoring, including detection of low abundance environmental chemicals and their metabolites. A number of databases and online tools are available to perform mass spectrum searches, but simple database searches can result in a large number of false positives. Here we present a novel algorithm based on metabolic correlation-network modules, adduct and isotope patterns, elution characteristics, intensity profiles, and pathway information for computational prediction of metabolite identities and to help elucidate biochemical roles of features with no database matches based on their module membership. xMSannotator is dependent on R packages WGCNA, SSOAP, RCurl, R2HTML and the KEGG REST service. A multi-step scoring algorithm is used for assigning confidence levels (high, medium, low, and none) to chemical matches that includes correlation-network module identification using WGCNA and sub-clustering of each module based on retention time prior to chemical matching. Mass defect analysis is performed to identify isotopic clusters. Chemical matching is performed using adduct rules on sub-cluster (as defined by module and RT) basis and a score is assigned to each chemical ID based on correlation within ions matching the chemical, RT range, adducts, pathway evaluation, isotopic patterns and ratio checks for multimers, multiply charged ions, NOPS and H/C rules. xMSannotator is an R package that includes functions for querying KEGG pathway and compound databases using the REST service, querying user-defined data sources in ChemSpider (ACToR, ToxCast, BioCyc, etc.) using SOAP, and for computational prediction of metabolite identities based on the multi-step algorithm using chemicals in KEGG, HMDB, and T3DB databases. The performance of the scoring algorithm was evaluated using the previously published standard mixture dataset of 95 metabolites with three replicates (Daly et al. Bioinformatics, 2014) available at (http://mzmatch.sourceforge.net/MetAssign.php). Annotation was performed using the database of 15,229 chemicals in KEGG available as part of xMSannotator using the following adducts: ("M+H","M+H+NH4","M+ACN+2H","M+2ACN+2H","M+2H","M+NH4", "M+Na","M+ACN+H","M+ACN+Na","M+2ACN+H","2M+H","2M+Na","2M+ACN+H"). Overall, 221 chemicals were assigned medium to high confidence scores by xMSannotator, out of which 40 were true matches that were present in the standard mixture. In order to test the effect of database size and content on annotation results, the chemical database used for annotation was restricted to only those chemicals that are associated with one or more pathways in KEGG. This led to a 35.7% decrease (N=142) in the total number of medium to high confidence matches and only 1% decrease (N=39) in the number of true matches. These results suggest that the accuracy of the annotation algorithms is strongly affected by the size and the content of the chemical databases used for annotation. The annotation results were also compared against the MetAssign results using the same dataset. Although MetAssign identified higher number of true matches (65 out of 95 with a combined probability >80%), xMSannotator uniquely identified 6 chemicals from the standard mixture that were assigned low overall probability by MetAssign. These results suggest that complimentary scoring algorithms can improve the coverage of true matches. Future work will focus on improving the precision of the algorithm and evaluate strategies to reduce false positives and redundancies. Software for metabolite annotation using a scoring algorithm based on

correlation network modules, elution characteristics, chemical rules, and pathway information.

POSTER 332

An Autonomous Workflow for Untargeted Metabolomics Profiling

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The conventional mass spectrometry metabolomics workflow tends to be a two-step process. Data is collected at MS1 and then processed to find any differential features of interest. Data is then re-acquired and any MSMS information collected is used for metabolite identification and manual database searching. An autonomous workflow combines both MS1 and MS2 data collection within a single-injection and simultaneous data processing and metabolite identification bringing the overall process down from weeks to hours. This allows for a more efficient and streamlined approach to move the researcher from sample to biology in a timely fashion. Data from a Zucker rat study was processed as proof of principal through the autonomous workflow using XCMSPlus, a software exclusively built for use with TripleTOF® instruments. MS and MSMS data was collected in a single-injection on a TripleTOF® 5600+ system in data dependent mode (AB SCIEX). Serum from the Zucker rat model was taken from 7-9 week old lean (n = 10), fatty (n = 10) and obese rats (n = 10). Samples were methanol extracted to remove the protein, centrifuged and the dried extract diluted in 100µl of Water:Methanol (80:20). Data was processed in both pair-wise and multi-group job mode and MS2 data was compared versus the local database for metabolite identification. XCMSPlus outputs a results panel with an interactive workspace where each extracted ion chromatogram can be reviewed with for each significant feature. There is also an array of multivariate reports from principal component analysis to an interactive heat map with hierarchal cluster analysis. The principal component analysis allows users to define which scaling method is best for the data and see both scores and loadings plots with metabolite annotations. In the interactive heat map both EIC's and spectral plots are given for each features that is clicked on. This allows for a fast and efficient way to interact with the data and understand the information from the experiment. When MSMS data is used with the platform, the local MSMS database is searched to find, with user conformation the correct compound. The user conformation allows users to check and confirm the MSMS match before exporting the dataset to an excel spreadsheet. Interrogating the LC/MS data, it was observed that there were many lipids changes amongst the three groups of rats (lean, fatty and obese) as well as other small metabolites. With the use of MSMS data to confirm the matches a higher certainty is available to users. Combining the system with the high scan speed of the TripleTOF® 5600+ system allows this information to be integrated with the MS1 data experiment to reduce the project analysis time dramatically. The end results mean a confirmed list of significant metabolites. An autonomous workflow for untargeted metabolomics profiling allowing for simultaneous data processing and metabolite identification.

POSTER 333

LipifyTM: software for high throughput identification and semi-quantification of lipids from LC-ESI-MS/MS-based lipidomics studies

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During calendar year 2014, our laboratory performed approximately 1700 liquid chromatography-tandem mass spectrometry (LC-MS/MS)-based lipidomics analyses. Given this great need for lipid profiling of biological samples, we developed a software program to conduct both informed and high-throughput analysis of lipidomics data from untargeted LC-MS/MS-based lipidomics studies. The software, LipifyTM,

quickly identifies and quantifies lipids from LC-MS/MS data sets while providing an interactive graphical user interface for users to explore the data. The software is not based on simple library matching but instead employs rules for lipid fragmentation patterns that enable discoveries of new lipid species that are classified according to general characteristics. Targeted data analysis simply involves the user specifying an electrospray ionization mode, lipid common name (e.g. PE(16:0/18:2)), and associated charge carrier. The software searches the loaded data set for the specified lipid and returns the highest scored matches and associated evidence for the user to view. For untargeted data analysis, the user must load in a set of lipid targets, which may include a theoretical component (previously unobserved). The software iterates over each MS/MS spectrum to find the best lipid match(es). For both informed and untargeted data analysis, the software allows the user to select results in the output table, which triggers a graphical display of all observed and theoretical lines of evidence used for identification. Data processing typically requires 10,000 MS/MS scans, and allows for confident identification of lipid molecular species due to the visually accessible evidence (e.g. annotated MS/MS spectra and isotopic profile, extracted ion chromatogram (XIC), and mass error of measured precursor ion). An XIC of the precursor ion showing the location of the MS-level survey scan and LC peak apex limits misidentifications due to isobaric, non-baseline resolved lipid peaks, and the annotated MS/MS spectra contain highlighted ions characteristic of the lipid class and fatty acid composition allowing rapid examination of the spectra. In addition, visualization of the XIC enables identification of lipid isomers within a single LC run. The software also allows for increasing the number of results per scan for the identification of co-eluting lipid species (e.g. PC and PE lipid subclasses). To date, LipifyTM has been used to process lipidomics data for a variety of sample types, from mammalian cells and tissues to cyanobacteria and fungal communities. The analysis of each sample typically results in coverage of multiple lipid categories (e.g. glycerolipids, glycerophospholipids, and sphingolipids) and lipid subclasses (e.g. triacylglycerols (TG), glycerophosphocholines (PC), glycerophosphoinositols (PI), and sphingomyelins (SM)). The exported output table contains the associated selected lipid common name and Lipid Maps nomenclature; the exact and observed m/z; retention time; instrument scan numbers corresponding to the LC peak apices and MS/MS events, LC peak apex intensity, and associated database identification numbers (i.e. LIPID MAPS PubChem, KEGG, InChi, HMDB). To demonstrate the utility of the software we will show results from a time series experiment of virus infected cells, where over 400 unique lipid species have been identified. Rapidly search an LC-MS/MS file against a database of >20,000 lipids, revealing multiple lines of evidence for a confident identification.

POSTER 334

OWL Stat App - An interactive web-application for univariate and multivariate metabolomics data analysis

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Metabolomics research has evolved considerably, particularly during the last decade. Over the course of this evolution, the interest in this ‘omic’ discipline is now more evident than ever. However, the future of metabolomics will depend on its capability to find biomarkers. For that reason, data mining constitutes a challenging task in metabolomics workflow, being a time-consuming issue which usually requires detailed knowledge of bioinformatics, statistics and specialized software. We have developed OWL Stat App, an easy-to-use web application for metabolomics data analysis. It combines powerful univariate and multivariate data analysis with pathway mapping tools and visualization capacities to facilitate interpretation of the results. OWL Stat App is a Shiny-based Web application, accessible independently of the operating system and without the need to install programs locally. It has been implemented entirely in the R language (R v.3.1.1; R Development Core Team, 2011; http://cran.r-project.org). All calculations are performed with caret package to classification training and ROCR package for visualizing classifier performance in R. The pheatmap package is used for drawing heatmaps. This application combines the R-based analytical tools with metabolite identification and pathway mapping tools, overlaying the users data on the pathway mapping libraries of SMPDB (The Small Molecule Pathway Database) and pathway libraries originally developed in our laboratory. Data handling is a challenge and one of the biggest

bottlenecks of the metabolomics workflow, but also the clue to accomplish valuable results. The advantages of using both univariate and multivariate approaches in data mining are clear, as both approaches are complementary and their results do not necessarily coincide. Then, we have developed OWL Stat App, an easy-to-use web application which facilitates the use, visualization and interpretation of univariate and multivariate data analysis. OWL Stat App has been developed in the R language and uses Shiny package, the web application framework for R. The available univariate statistical tests and visualization tools include a heatmap and an interactive volcano plot. By clicking on a metabolite of interest on the volcano plot, a description according to The Human Metabolome Database (HMDB) and Kyoto Encyclopedia of Genes and Genomes (KEGG) is provided, as well as a boxplot, histogram, density and Normal Q-Q plots, Shapiro-Wilk Normality Test, Student's t-Test (or Welch´s t test if unequal variances), outlier Analysis (following Chauvenet's criterion), fold-change and ROC analysis. All mentioned statistical tests and graphical outputs can be recalculated applying different transformations to the variables (y=x; y=x2; y=x1/2; y=1/x; y=1/x2; y=1/x1/2; y=log(x+1) and y=sh(x). In order to help in the interpretation of the metabolite's changes in a biologically meaningful context, this app is linked to the pathway mapping tools of SMPDB and to pathway graphical outputs originally developed in our laboratory. OWL Stat App also includes the following multivariate data analyses: principal component analysis (PCA); the study of the correlation between samples; a heatmap per sample, showing the relationship among the samples according to the metabolite levels and the hierarchical clustering. The web application is platform independent and has been successfully tested on Google-Chrome 41.0, Mozilla Firefox 36.0.1, Safari 5.1.7 for Windows and IOS 8.1.3. OWL Stat App is a web application for metabolomics univariate and multivariate data analysis with visualization capacities to facilitate interpretation.

POSTER 336

R2D2web, a Comprehensive GC open software and colaborative database to analize metabolomic data from human biofluids

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GCxGC-TOF is increasingly being used by metabolomic researchers due to its peak capacity and resolution. Nevertheless, a complete, open-source software package to identify and quantify reliably the metabolome of biofluid samples such as serum or urine remains an open field of research and development. With this contribution, a web version of the R2D2 software will be presented. The package will be able to process netcdf files and build up a metabolite database using the colaborative effort of the metabolomics community through a web page interface. The use of standard operation procedures optimized for each biofluid will garantee the interoperability between labs of the database and the optimization of the measurement procedures. R2D2web is programmed using the free and open statistitcal platform R and the shiny SDK to make it accessible through any web browser. A GCxGC and MS optimized optimized method for human serum and urine will be posted at the R2D2web URL as soon as all the trials are finished and a final method for each biofluid has been tested.To use the software an initial subscription (username and password) will be necessary. The software will keep track of each user contribution and will require each user to strictly comply with the guidelines to upload measurements for library inclusion. The software package and the library will be downloadable to execute locally for faster operation. R2D2web is a software developed in the R statistical programming platform. The main code is written in the R language. Thanks to the Shiny package, the resulting code can be accessed remotely or locally using a standard web browser and, therefore, it is platform independent. The first version of the core software is capable of detecting, integrating and identifying peaks from serum samples. So far, identification is made possible by matching each peak spectra to the GOLM metabolite database. This open-source database is ideally suited to the use of a Time Of Flight Mass Spectrometer, being far

more accurate at matching than the NIST library which is more suitable to standard Quadrupole Mass Spectrometers. Further work is being conducted to include the absolute and relative retention time using the standard FAMES as a reference. In this way, there will be two criteria to refine and enable automatic metabolite identification. A registered user will be able to upload information about new metabolites if he/she has followed the chromatographic and spectrometry methods described in the website for each different biofluid matrix, the first being serum. He will input the standard's name, CAS number, concentration and absolute retention times of each compound's peak. The software will automatically compute the spectra and relative retention index based on the standard FAMES added during the derivatisation process and the peak's area. Progress is being made in 2D deconvolution, baseline correction and line-shape fitting for more accurate quantificication. If different concentrations of each metabolite are added, a specific metabolite calibration curve will be derived. Easy web applications in R Shiny is an open source R package that provides an elegant and powerful web framework for building web applications using R. Shiny helps you turn your analyses into interactive web applications without requiring HTML, CSS, or JavaScript knowledge GCxGC-TOF open source software, Retention index based database, Colaborative effort database building, specific databases for different human biofluids

POSTER 337

OpenMSI: A web-based portal for rapid processing of size-independent, next-generation mass spectrometry imaging experiments

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Mass spectrometry imaging (MSI) enables researchers to probe molecules directly within the architecture of the biological matrix. Unfortunately, efficient exploration, analysis, and management of data from MSI experiments are persistent, major challenges to this rapidly developing field. Despite the availability of numerous dedicated file formats and software packages, it is a widely held viewpoint that a major challenge is simply opening, analyzing and sharing a file in a timely manner without loss of information. Locally available compute resources are often insufficient to store and process the amount of MSI datasets generated, and high-performance computing resources are needed. To overcome current bottlenecks and position the field for next-generation mass spectrometers and imaging systems a new approach is required. We present OpenMSI, a web-based science gateway and analysis framework that addresses these challenges via an advanced, high-performance, extensible file format and web API for remote data access. OpenMSI (http://openmsi.nersc.gov) is hosted at the National Energy Research Scientific Computing center (NERSC). This supercomputing facility provides easily integrated computing resources, high-performance file systems, and web-services that can be coherently joined to build effective solutions to scientific data-centric challenges. The OpenMSI file format enables storage of raw MSI data, metadata, and derived analyses in a single, self-describing format based on HDF5 and supported by a large range of analysis software (e.g. Matlab and R) and programming languages (e.g., C++, Fortran and Python). Careful optimization of the data layout accelerates common, selective data access operations for retrieving images, spectra and data subcubes by up to several orders of magnitude while minimizing data storage requirements. Analyses and data from other imaging modalities can be easily integrated with the file format and API. To make remote high-performance compute resources accessible for analysis and to facilitate data sharing and collaboration, we describe an easy-to-use yet powerful web API, enabling fast and convenient access to MSI data, metadata, and derived analysis results stored remotely. The OpenMSI file format and web API enable fast and easy access to MSI data and straightforward integration of MSI technologies with modern analysis methods, web technologies, and client-side libraries. Data layout optimization using chunking, compression, and data replication were found to be critical enablers of rapid data access resulting in a >2000-fold improvement in image access. The OpenMSI platform enables data retrieval speeds of images and spectra of less than 0.3s across the

Internet even for 50GB MSI datasets. OpenMSI enables the analysis, visualization, and sharing of huge datasets using web-based tools.

POSTER 338

R-cloud based analysis of metabolomics datasets with MetaboAnalyst in MetaboLights Labs

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MetaboLights has been widely publicised and recognised throughout the community as the first general purpose, cross-species, cross-application database for metabolomics, since its launch in 2012. The archived raw data and various reference data within MetaboLights is continuing to grow rapidly. Despite significant improvements in software for metabolomics analysis, these tools are in general, fragmented across laboratories or specific to a particular workflow. To address this we are in the process of collecting open access tools and investigate cloud-based workflows for the most common approaches used in metabolomics, acting as a portal. We are in the process of implementing 'MetaboLights Labs', which will be a online staging area for all these various analysis tools and facilitate cloud based data analysis. MetaboAnalyst 3.0, is a widely used popular web-based tool for stepwise metabolomic data analysis. R-Cloud is a scalable, distributed framework to expose R to java applications, also a general resource pooling framework suitable for dispatching compute-intensive tasks to the large computing cluster at EMBL-EBI. We are currently integrating Metaboanalyst 3.0 into 'MetaboLights Labs', coupled to the powerful R-Cloud cluster in the back-end for compute intensive analysis. MetaboAnalyst 3.0 has been designed to support robust statistical analysis, data exploration, data visualization, as well as functional interpretation of metabolomic data. All of these latest available analysis modules are configured to run remotely on a distributed R-cloud cluster at the European Bioinformatics Institute. From within the 'MetaboLights Labs' framework, Metaboanalyst modules can be used to analyse user's own datasets or any public metabolomic datasets from the MetaboLights Archive. The analysis results can be stored in the user workspace and are downloadable. The R-cloud framework assists in handling the data-intensive analysis and the heavy user load should it arise. Free and open comprehensive analysis tools suite greatly enhance the utility of MetaboLights for the metabolomics community.

POSTER 339

Making Metabolomics More Meaningful – Introducing MetaboAnalyst 3.0 and MetabolomeNet

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Since its first launch in 2009, MetaboAnalyst has experienced more than a 50X growth in user traffic with currently over 45,000 jobs processed per month. To keep up with the rapidly increasing computational demands and a growing number of requests to support translational applications, we performed a substantial rewrite and major feature upgrade of the tool. MetabolomeNet is a novel systems biology tool designed to address the statistics and visualization challenges in analyzing quantitative metabolomics data based on global metabolic networks. The tools are implemented using open source languages – R for statistical computing, Java for web application framework and JavaScript for interactive graphics. To take advantage of the high-performance cloud-based computing platform, we are currently testing the Google Computing Engine to host our applications. We have completely re-implemented the MetaboAnalyst tool suite using the latest web technologies, which have substantially improved its performance, capacity and user interactivity. Three new modules were added, including: 1) a module for biomarker analysis and new sample prediction; 2) a module for sample size estimation and power analysis; and 3) a module for integrative pathway analysis. MetabolomeNet integrates statistics, pathways analysis and topology analysis within the global metabolic networks. Various functions are

implemented allow users to identify novel connections, topological changes, and modules that are associated with different conditions. All results are presented in a highly interactive visual analytics environment. Integrating robust statistics, high-performance visualization technolgogies and network biology for improved understanding of metabolomics data

POSTER 340

A GALAXY WORKFLOW FOR METABOLOMIC NMR DATA PRE-PROCESSING

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High-throughput technologies such as Liquid Chromatography coupled to Mass Spectrometry (LC-MS) or Nuclear Magnetic Resonance (NMR) generate huge amounts of data. These call for high-performance and user-friendly analysis tools for experimenters. We have recently developed Workflow4Metabolomics (W4M) [1; 2], a virtual research environment (VRE) built upon the Galaxy web-based platform technology. W4M (http://workflow4metabolomics.org/) currently contains pre-processing tools for LC-MS metabolomics data. In this work, we describe two new modules for the bucketing and normalization steps of NMR data pre-processing. size bucketing and peak integration. The trapezoid method was used to compute the area under the curve in each bucket. The second module offers several methods for the normalization of profiles, including Probabilistic Quotient Normalization [3] and normalization by the total intensity, or by a quantitative variable provided by the user (eg, weight, osmolality). Second, we validated these functions on a dataset consisting of 44 samples from a study addressing the toxicity of bisphenol A in the mouse [4]. The samples were analysed on a Bruker DRX-600 Avance NMR spectrometer operating at 600.13 MHz for 1H resonance frequency. The raw spectra were Fourier transformed, phase and baseline corrected, and calibrated using the manufacturer software. The spectra were then binned and normalized using our tools, and the results were compared with the commercial software AMIX V3.9.11 (Bruker, Rheinstetten, Germany). Third, we integrated the two modules into W4M. A web interface with a whole of parameters was designed for each R function. In addition, the dataset is publicly shared on W4M and can be used for training. Our modules provide new pre-processing functionalities for NMR spectra on W4M, which can be combined with the existing statistical modules.

POSTER 341

METASPACE: A new European project on cloud computing for spatial metabolomics

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Metabolomics is recognized as a crucial scientific domain, promising to advance our understanding of cell biology, physiology, and medicine. Metabolomics complements genomics, transcriptomics, and proteomics by analyzing the final read-out of biochemical processes and by revealing the contributions of non-genetic factors, such as the environment, diet, or microbiome. In the last years, metabolomics progressed from simply cataloguing chemical structures to answering complex biomedical questions and enabling new discoveries in life science. This progress has been made largely in the analysis of liquid samples, so the next frontier for metabolomics now lies in spatial metabolomics, where the challenge is to localize hundreds of metabolites directly from biological tissue sections with cellular and sub-cellular spatial resolution. Our European project, starting in July 2015 and uniting 8 partners from 6 countries, aims to enable untargeted spatial metabolomics for translational research and clinical applications by

providing novel bioinformatics tools. We will develop novel algorithms for the high-throughput metabolic annotation of High-Resolution imaging Mass Spectrometry (HR imaging MS), turning the big data generated into molecular knowledge. We will consolidate these algorithms into an approach for interpretation of candidate molecules in light of existing molecular knowledge bases, particularly metabolic pathways. We will implement an open online engine METASPACE aimed at supporting research and clinical labs in performing spatial metabolomics studies that will be released under a permissive open-source license allowing its use both in academia and industry. The project was funded by the European Commission, in the Horizon 2020 program, subprogram Personalizing Health and Care. The project will start in July 2015 and will run for 3 years. We plan substantial outreach activities to ensure constant communication and interaction with the mass spectrometry and metabolomics communities. Our Advisory Board includes toPoster level experts from academia (metabolomics, clinical metabolomics, imaging mass spectrometry, bioinformatics, open science) as well as mass spectrometry vendors, pharmaceutical industry and editors of mass spectrometry and metabolomics journals. We already agreed on collaborating with other related large-scale projects (COSMOS, 3D-MASSOMICS), initiatives (Metabolomics Society, ELIXIR, COST Action BM1104, Imaging Mass Spectrometry ESMI Study Group, Computational Mass Spectrometry HUPO/ISCB interest group) and resources (MetaboLights and GnPS repositories) and are looking for opportunities to get into agreement with other initiatives. We will present the scientific plan of the project as well as the preliminary results with the aim to collect feedback from the community. Our European project aims at creating an open online engine for spatial metabolomics.

POSTER 342

Untargeted LC-MS data post-processing: Look at only the peaks that matter!

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Untargeted metabolomics by high-resolution accurate-mass LC-MS generates large data sets containing metabolic features and isotopes, adducts and in-source fragment ions. Managing such data sets in a concise and streamlined manner is a bottleneck for any metabolomics laboratory. There are many software programs, commercial or freeware, available for generating pliable data tables which can be exported to a cell-based program for rudimentary statistics or further exported in table form to other statistical or network mapping programs. However, it is tedious and time consuming to curate an untargeted dataset manually, by combining multiple ion-adducts generated from the same metabolite, removing duplicate peaks, or isotopic peaks to form a concise peaks table which can be analyzed for statistical significance. The LC-MS data “Post Processor Tool” uses a variety of features to shape a raw data set into a more concise and statistically powerful dataset which is ready to be further analyzed. These features are easily adjusted by the user to mold them to fit a variety of data acquisition platforms. The Post Processor Tool offers a variety of features that can be used separately or together including; Duplicate Peak Filter, Isotope Matching, Adduct Joining, Removal of Contaminant Ions and Peaks Table Formatting. Lipidomic profiling via LC-MS was performed on 100 blood plasma samples and 15 QC samples which were then processed by MZmine. This data set was then submitted to the Post Processor Tool for further data curation. Adduct Joining determined correlations of specific, ion adducts in the data set. These ion adducts were defined by the user, allowing any mass difference to be searched for correlation. Adduct joining utilized user defined mass and retention time tolerance parameters, to combine or flag potential adducts for review. These parameters tell the Post Processor Tool the name of the ion-adducts to join, the accurate mass difference and the minimum R2 value to recognize adducts, as well as the R2 value at which to automatically join features. The user defined values for mass accuracy tolerance (0.01 m/z), and retention time tolerance (0.02 min) were the same values used for both adducts and isotopes. Additionally, the user defined adduct specific information for all adducts desired, such as [M+H],[M+Na],21.9787,0.0,0.8 and [M+NH4],[M+Na],4.956,0.0,0.8. Using the data set described above, which contained 2848 features, 105 adducts were automatically matched. An additional 88 matches were flagged for human review, of which 40 were approved and resubmitted to the program to automatically

join. Duplicate Peak Removal flagged peaks for user review that may have been incorrectly aligned as separate peaks by the data processing software. Utilizing user-defined settings for peak similarity based on retention time variance, mass accuracy variance, and similarity of peak heights; 57 potential duplicate peaks were found in the sample set, 33 matches were more than 60% similar. Isotope Matching searched the entire dataset for isotopic relationships between features with close retention times and appropriate accurate masses. A user defined R2 value representing the correlation of each peak to its isotopic partner. The user defined an R2 of 0.8 for this data set. Isotope matching flagged 227 prospective isotopes for user review of which 121 were confirmed. Removal of Contaminate Ions allows the user to instantaneously remove known systematic contaminate ions. This tool allows users to quickly and effectively curate .csv files, saving time and enhancing power in statistical analyses.

POSTER 343

Characterization of Differential Metabolites in Bacteria Using a Q-TOF LC/MS Based Metabolomics Batch Data Analysis Workflow

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The growth of bacteria has been studied for more than a century. It was found that cells remain alive and metabolically active during stationary phase, but often respond differently to stimuli or stresses than during growth phase. Researchers hypothesized that one or more metabolites were produced during stationary phase that rendered a bacterium susceptible to killing or, alternatively, that a protective metabolite was lost during stationary phase. To our knowledge, there was no information available about the characteristics of those metabolites. We present a Q-TOF metabolomics approach to characterize bacterium metabolites. The use of a suite of batch, data processing software made data analyses more efficient and automated, which enables rapid transformation of complex raw data into biologically relevant information. Cultures were harvested either in the growth phase or in the stationary phase, rinsed twice with phosphate buffered saline to assure removal of any residual extracellular media, then homogenized and extracted. LC/MS Metabolomics workflow involves 1) acquire positive and negative ESI data in TOF mode from the extracts of growth and stationary phases 2) find features from raw data using an untargeted batch feature extraction algorithm, 3) perform differential analysis and annotation using a multivariate statistical analysis software, 4) conduct targeted MS/MS analysis of the differential features of interest using Q-TOF LC/MS, 5) use molecular structure analysis software to aid structural identifications of metabolites based on MS/MS spectra matches and databases searching. A Q-TOF LC/MS workflow was developed to investigate differential metabolites of a bacterium in the growth phase versus the stationary phase. Chromatographic separation used an aqueous normal phase (ANP) column to maximize polar metabolite coverage. Metabolites were detected by high resolution MS analyses in positive and negative ion modes respectively. Batch feature extraction software was used to enable automated feature finding from large complex raw data. As a result, 488 features from positive ion data and 623 features from negative ion data were found based on user-defined compound filtering criteria and manual curation. Those features were then assessed using a Filter on Volcano Plot algorithm with a cutoff of P smaller than 0.005 and fold change greater than 2. The statistical results yielded features whose abundance differed significantly in the growth phase versus the stationary phases. For the positive ion data, 98 of 488 features displayed statistical significance, 57 of them were found to have higher abundances in the growth phase compared to the stationary phase. Likewise we detected 152 of 623 features are significantly differential in the negative ion data and 52 of them have higher abundance in the growth phase than in the stationary phase. The next step was annotation and identification of those differential features with the goal to understand the biological and biochemical context of the metabolomics data. We will demonstrate how molecular structure analysis software can rapidly facilitate annotation and identification of over 100 differential features, using the accurate mass of precursor and fragment ions, isotopic patterns, databases searching, and accurate mass MS/MS library matching. A batch data analysis workflow for characterizing bacterium metabolites acquired on a high resolution Q-TOF LC/MS.

POSTER 344

Towards the Development of a Factor Analysis Based Peak Finding and Alignment Algorithm for Raw GC-TOFMS Metabolomics Data

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Gas chromatography (GC) and comprehensive two-dimensional GC (GCxGC) coupled to mass spectrometry are powerful tools for metabolomics studies. These approaches provide information on individual analytes and metabolites and generate a wealth of data to describe complex samples and investigate important metabolic questions. Fundamentally, complex samples are converted into complex data and the challenge of extracting useful information often remains. This challenge is compounded as sample complexity and sample set size increases, both of which are common in metabolomics studies. One step towards extracting information is the consistent alignment of features (i.e., analytes) across all of the samples. Accomplishing this alignment prepares the data for further comparisons and differential analyses to determine important metabolic differences within the complex samples. A factor analysis based method is being developed to consistently align, find peaks, and deconvolute coelutions within GC and GCxGC-TOFMS data sets. The raw GC (or GCxGC) data, including the first (and second, if applicable) dimension retention time, mass spectral, and sample dimensions are compiled prior to factor analysis. The method provides concentration and spectral information for each factor without enforcing multi-linearity or requiring the number of factors to be specified in advance. The factors are rotated and qualified to determine those that describe chemically relevant chromatographic signals. The qualified factors (deconvoluted spectra) are library searched and the concentration information is combined to an aligned peak table for further data analyses. Preliminary data are shown that demonstrate how factor analysis of the raw data directly provides alignment, deconvolution, and peak finding for GC (and GCxGC-TOFMS) data sets. Factor analysis yields individual factors (intensity and spectral information) that describe the entirety of the data. Some of these factors describe noise and nonlinearities while other factors describe chemically relevant chromatographic signals. Peak finding is accomplished by qualifying the factors that describe chemically relevant chromatographic signals compared to those that describe noise. When the data contain variations in chromatographic retention, mass spectral patterns, or expression across the sample dimension, these differences are captured by the factors, effectively deconvoluting chromatographic coelutions. Alignment is also achieved as each factor describes the same information across all of the samples. Utilizing the raw data provides benefits over approaches that perform initial peak finding with deconvolution and then align the Peak Table information, particularly in cases of complete chromatographic coelution and in cases where sample to sample variations led to differences in the initial peak finding results per sample. When raw data are leveraged with all of the samples together, as they are here, peak finding and deconvolution can be more consistent because all of the data are considered simultaneously. Additionally, the differential expression of analytes between samples provides another dimension of information to improve deconvolution. This approach to peak finding, deconvolution, and alignment provides better consistency of the information across all samples and metabolomics examples are demonstrated. A factor analysis approach is demonstrated that finds peaks, deconvolutes, and aligns GC and GCxGC-TOFMS metabolomics data sets.

POSTER 345

A Capability for Routine High Resolution Spectra Enhances the Scope of Smart Elemental Composition

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The routine and easy ability to collect LC-MS data at resolutions higher than 100,000 means that even out to mass 500, multiple An (n>0) isotopes are resolved. We describe here a fast and accurate method to identify these isotopes. Once identified, the presence and intensity of isotopes other than Carbon (exotic isotopes) can be used to limit the elements in use and the abundance of each element in and elemental composition calculations. Applying such limits can have a dramatic effect in speeding up the calculation – why bother including Sulphur in the elemental composition simulation of a compound when the high resolution data shows it contains no Sulphur? We locate Carbon isotope chains in each scan. High-scoring patterns are then subjected to chromatographic feature detection. After the feature detection phase, exotic isotope clusters (13C isotope chains that contain an exotic element) are matched to their corresponding Carbon isotope clusters by chromatographic lineshape matching. To increase the sensitivity of this method, low-intensity ions that lack isotopes are also feature-detected so that they might be correlated with another Carbon isotope cluster. Ions that lack isotopes, but are sufficiently intense that they should show some are ignored. After this step, full elemental composition is performed for each component of interest, using the knowledge obtained on exotic element abundance for that component. We locate Carbon isotope chains in each scan, by looking for ions that are separated by 1.0033/n Da (n is the charge) If the charge is not obvious, we look at all charge states 1-6, for these patterns. The intensity of each An (n>0) must be no less than predicted by the mass and the 13C abundance; we calculate a pattern match score based on a mass and intensity fit to a predicted 13C isotope chain (without penalizing An isotopes for being too intense). High-scoring patterns are then subjected to chromatographic feature detection. After the feature detection phase, exotic isotope clusters (13C isotope chains that contain an exotic element) are matched to their corresponding Carbon isotope clusters by chromatographic lineshape matching. To increase the sensitivity of this method, low-intensity ions that lack isotopes are also feature-detected so that they might be correlated with another Carbon isotope cluster. (This is due to a common occurrence for low abundance species, that the 34S or 18O A2 isotope is visible but the A3 is of too low intensity to be seen.) Ions that lack isotopes, but are sufficiently intense that they should show some are ignored. After this step, and any grouping of adducted species and removal of background features, full elemental composition is performed for each component of interest, using the knowledge obtained on exotic element abundance-for that component- to limit the elements and abundances used in the calculation. We will show multiple examples of this technique applied to several high-resolution data files containing mixtures of small molecules – drugs, pesticides, contaminants. Elements other than Carbon are easily identified in high-resolution LC-MS data without time-consuming elemental composition calculations.

POSTER 347

Global Optimized Targeted Mass Spectrometry in Metabolomics

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Mass spectrometry (MS) is an essential tool in metabolomics for investigating alterations in metabolism and metabolic pathways in biological systems. Global profiling and targeted detection are the two most important methods in MS-based metabolomics. However, neither is ideal, and they have somewhat complementary advantages and disadvantages. Global profiling has wide metabolite coverage, but it often suffers from missing values, poor quantitation, and complicated data processing. Targeted MS has the significant advantages of high data quality, great selectivity, and excellent quantitation; however, a major limitation of targeted MS in metabolomics is its limited metabolite coverage. Therefore, there is a high demand in metabolomics to improve these detection technologies towards better qualitative and quantitative capabilities. In this study, we have developed a new approach, Global Optimized Targeted (GOT)-MS, with the advantages of both targeted detection and global profiling. GOT-MS was developed based on a single liquid chromatography triple quadrupole (LC-QQQ) mass spectrometer in both positive and negative ionization modes. Aqueous metabolites were extracted from a serum sample and separated using hydrophilic interaction liquid chromatography (HILIC). The key step in GOT-MS is a global search of precursor and product ions in order to optimize the detection capabilities of LC-QQQ, which was performed in the mass range of 60-600 Da. The multiple reaction monitoring transitions (MRMs) with good peak shapes and high signal-to-noise ratios (S/Ns) were selected and optimized to obtain optimal

MS parameters. We first performed selected ion monitoring (SIM) incremental scanning to take advantage of its high sensitivity and good S/N. Given the unit mass resolution of the MS quadrupole, we used an m/z increment of 0.5. We examined each individual SIM, and the m/z values that produced good peak shapes and S/Ns were selected as precursor ions. We then carried out tandem mass spectrometry (MS/MS) scanning with incremental collision energy (CE) to profile product ions. Three CE values were selected: 5 V, 20 V, and 35 V. Most metabolites will fragment under these CEs, and MS/MS spectra under CE of 5 V provide more accurate m/z values for the precursor ions (e.g., 60 could be updated to be 60.1). With both precursor and product ions available, MRM scanning was used to optimize the fragmentor voltage, cell accelerator voltage, and CE. In the analysis of human serum, 639 GOT-MS precursor ions and 2020 MRMs were determined from the peaks with decent peak shapes (based on symmetry, peak width, etc.) and S/Ns. Notably, scheduled MRM scanning was used to measure >500 MRMs in a single injection (9 min separation). Although definitive identification awaits further confirmation, metabolite candidates for ~50% of the GOT-MS MRMs could be discovered in the Metlin database. GOT-MS was designed to essentially optimize the detection performance of a single LC-QQQ mass spectrometer, and given the capabilities of QQQ-MS, it should be very selective and highly reliable for quantitative analysis. Because precursor and product ions are globally searched, GOT-MS is applicable for a wide range of metabolic studies to detect not only well-known metabolites but also unknowns. LC retention and fragmentation patterns are useful to resolve the metabolites with similar molecular weight. Faster and more sensitive QQQ instruments will detect even more species. GOT-MS integrates the advantages of both global profiling and targeted detection, including unknown detection, excellent quantitation, and good reproducibility.

POSTER 348

Multivariate Curve Resolution as a feature selection tool for metabolomic studies

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Multivariate Curve Resolution (MCR) is a popular chemometric method which decomposes a data matrix into their factor matrices according to a bilinear model under natural constraints. These two-factor matrices are related, respectively, to the concentration and spectra of components present in the investigated system. MCR is especially suitable for the resolution of chemical constituents in complex mixtures, and one of its main advantages is the flexibility that allows its application to different types of studies and instrumental techniques. In the case of metabolomics studies, the MCR chemometric method can be extended to the evaluation of the changes in the metabolites concentration among samples due to external stressing factors. In this work, the reliability of the MCR-based approach was tested using data from two metabolomic mass-spectrometry based platforms. In a first example, the MCR analysis of capillary electrophoresis – mass spectrometry (CE-MS) data was applied to the study of changes in the yeast metabolome induced by temperature changes. Challenges due to the variability in migration time shift due to changing capillary conditions were especially relevant, in this case. In a second example, MCR was applied to MS imaging (MSI) data consisting of a set of MS images of rice plant leaves exposed to different concentrations of heavy metals. In this case, the major challenge lay in the simultaneous analysis of multiple MS images. Application of the MCR method provided satisfactory results in the two considered examples. In both cases, the analysis workflow started with the compression of the data dimensionality by a reduction of the spectral information without losing mass resolution. In the example of the CE-MS data, MCR simultaneous analysis of control and treated samples allowed the determination of more than 50 potential metabolites involved in yeast metabolism. Each one of these metabolites was tentatively identified by its resolved high-resolution mass spectrum, and at the same time, it was also characterized by its migration time. In this case, significant shifts in the migration times could be observed between samples but this was not a major drawback for the application of MCR and proper resolution of candidate metabolites. In the example of MSI data, each experimental data cube (size of x-pixels, y-pixels, m/z values) of the considered samples was unfolded into 2D matrixes (size of x-pixels by y-pixels, m/z values) and analyzed simultaneously by the MCR method. In this case, one of the

factor matrices contained spectral information (as in the example of CE-MS data) and the other contained the information regarding the presence and relative amounts of the resolved image constituents on the surface of the sample. Refolding of this single component data matrix allowed obtaining the distribution maps of the different metabolites from which their variations among samples could be investigated. Multivariate Curve Resolution is proposed as a feature selection tool in metabolomic studies using different analytical platforms.

POSTER 349

PITracer: An R package for Untargeted Metabolomics Studies by Tracing Pure Ion Chromatograms in Liquid Chromatography/Time-of-Flight Mass Spectrometry-based Metabolomics Data

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Untargeted Metabolomics using LC/TOF-MS is powerful for detecting and quantifying a broad range of small molecules in complex biological fluids. However, detecting the ion traces produced by metabolites in complex biological samples with LC/TOF-MS is still a major challenge for untargeted studies since majority of ions are not the products of metabolites. In this work, we developed PITracer, an R package for detecting the ion traces produced by metabolites contained in complex biological samples to generate pure ion chromatograms (PICs) according to the distribution of the m/z values of ions. As a result, PICs can be generated for more precise chromatographic peak detection when compared to current algorithms for current LC/TOF-MS-based untargeted metabolomics studies, e.g. centWave and MZmine. PITracer comprises four major steps to extract PICs and to identify peaks in the PICs. In the first step, the relative mass difference (RMD) tolerance is estimated based on the distribution of RMDs between the adjacent m/z values in a sorted m/z value list. In the second step, the most present base ion would be extracted as the reference ion to calibrate the m/z values to reduce the RMDs. In the third step, the PICs are extracted according to the estimated relative mass tolerance of the calibrated LC/TOF-MS profile. Finally, the peaks in each PIC would be detected recursively and the m/z values of the detected peaks will be corrected according to a known (user-specified) metabolite in samples. PITracer, an R package for pure ion detection, was developed to generate the pure ion chromatograms and detect chromatographic peaks produced by metabolites in LC/TOF-MS-based metabolomics data. PITracer estimates the relative mass tolerance for each LC/TOF-MS profile, considers the RMD tolerance of saturated chromatographic peaks, and uses the most often present m/z value in base ions to calibrate m/z values to reduce the RMDs and improve mass precision. The pure ion chromatograms are extracted according to the estimated relative mass tolerance with the calibrated LC/TOF-MS profile for peak detection algorithms. The m/z values reported by the peak detection algorithms can also be corrected additionally according to a user-specified metabolite. PITracer was evaluated using two data sets containing 373 human metabolite standards, including 5 saturated standards considered to be split peaks resultant from huge m/z fluctuation, and 12 urine samples spiked with 50 forensic drugs of varying concentrations. Analysis of these data sets show that PITracer correctly outperformed existing state-of-art algorithm and extracted the pure ion chromatograms of the 5 saturated standards without generating split peaks and detected the forensic drugs with high recall, precision, F-score, and small mass error. PITracer can extract the chromatograms with varying m/z value fluctuations and detect analytes having low intensities with sensitivity and precision. Hence, analyzing LC/TOF-MS-based metabolomics data with PITracer can detect the metabolites more thoroughly and might reveal new metabolites. PITracer allows for the full analytical power of LC/TOF-MS to be utilized for automated exploratory analytical chemistry in metabolomics studies. PITracer is a tool for untargeted metabolomics analysis that can extract ions generated by real metabolites more accurately.

POSTER 350

Tools for network-based analysis and visualization of lipidomics data

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Development and application of high-resolution analytical methods has considerably advanced metabolic profiling of diverse lipids in complex biological samples. However, the development of computational data analysis tools still lags behind. Pathway mapping techniques commonly used for secondary analysis of metabolomics data usually fail when it comes to analyzing lipids, primarily due to insufficient coverage of lipid metabolism by most pathway databases. We present here our new correlation-based method for building metabolic networks that does not rely on prior knowledge of metabolic pathways, and a new version of our previously developed tool Metscape (http://metscape.ncibi.org) that supports building and visualization of correlation networks, and demonstrate their application to the analysis of lipidomics data. Pearson's correlations can be used to establish linear marginal associations between lipids, but they do not differentiate between direct and indirect associations. Partial correlations can identify conditional dependencies, but they require the number of samples to be greater or comparable to the number of metabolites. Taking into account sparse structure of biochemical networks, partial correlations can be calculated using regularized estimation techniques. We developed a new method that uses the De-sparsified Graphical Lasso (DGlasso) modeling procedure to identify connectivity among large numbers of metabolites using fewer samples. DGlasso is implemented as part of our CorrelationCalculator program available at http://metscape.ncibi.org. The analysis results can be visualized using Metscape, a plugin for the widely used network analysis and visualization tool Cytoscape. Using Metscape with lipidomics data After importing a list of lipids quantified across multiple samples into the CorrelationCalculator, users are given options to perform basic normalization procedures, Pearson's correlation, basic partial correlation and DGlasso partial correlation. The program provides several choices for visualizing the results, including static heatmaps and data export for interactive heatmap exploration. The results can also be downloaded or sent directly to Metscape. Within Metscape users can explore resulting networks, taking advantage of a broad range of features built into the program. Some examples include visualizing the differences in metabolite concentrations and significance levels and exploring the relationships between different classes of lipids. Applications To validate our method and to demonstrate the utility of the new Metscape module we used a dataset generated via shotgun lipidomics approach developed at the Michigan Regional Comprehensive Metabolomics Resource Core (MRC2). Lipid profiles were obtained using serum samples from patients with mild to moderate chronic kidney disease (CKD) predictive of progression to end stage kidney disease (ESKD) in Chronic Renal Insufficiency Cohort (CRIC) patient population. We will show how our tools can be used to generate data-driven correlation networks for lipidomics data. Users have the ability to set correlation thresholds, import additional information (e.g. specify groups of metabolites) and interactively explore resulting networks. In addition, we will demonstrate Metscape filtering capabilities and other advanced features that provide an easy way to explore large networks. The program uses established lipid classifications (LipidMaps and LipidHome) to enable ontology-based data exploration. Finally, we will demonstrate Metscape workflows for augmenting data driven networks with biochemical pathway information to facilitate generation of biological hypotheses. We present a set of tools for data driven network analysis and visualization of lipidomics data and demonstrate their applications.

POSTER 351

Slow High Resolution-Magic Angle Spinning NMR for metabolomics studies on fragile tissues

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High-resolution magic-angle spinning (HR-MAS) nuclear magnetic resonance (NMR) spectroscopy is a powerful analytical tool for the study of complex biological systems, providing metabolic information on intact tissues with no need for sample extraction. However, the extreme centrifugal forces experienced with conventional spinning frequencies (several kilohertz) compromise the “non-destructive” nature of NMR, and hinder metabolomics studies of fragile samples. Performing HR-MAS analysis at moderate spinning rates (slow HR-MAS) makes it possible to preserve sample integrity. In practice, NMR spectra obtained at low spinning rates with current standard protocols are plagued with numerous spinning sidebands (SSB), compromising metabolomics applications. Here we introduce a complete protocol for the characterization of fragile tissues by slow HR-MAS NMR and demonstrate its potential for metabolomics studies. In this work, we first compare the effect of conventional (4 kHz) or slow (400 Hz) MAS on the integrity and metabolic profiles of two fragile samples: fish eggs and heifer liver. Then, we develop and apply on a heifer liver sample a robust preparation protocol leading to SSB-reduced spectra at low MAS rates, adapted to standard HR-MAS inserts and high magnetic fields (up to 700 MHz). Finally, in a metabolomics-oriented study, we compare the performance of conventional and slow HR-MAS for discriminating 30 liver samples from 3 groups of rats fed with different diets. The comparison between conventional and slow HR-MAS for several sample types shows that slow HR-MAS better preserves the integrity of fragile biological objects and limits the extraction of the metabolites from the samples. In contrast, severe sample degradation and metabolite release are observed when performing conventional HR-MAS experiments. These results demonstrate the high potential of using slow MAS for non-destructive HR-MAS studies on fragile samples such as tissues and biopsies. Using an optimized protocol for sample preparation, we obtain 1H NMR spectra that are free of spinning sidebands, which otherwise plague slow HR-MAS experiments. In order to remove unwanted phase distortion that occur at low MAS rates with the conventional Carr-Purcell-Meiboom-Gill (CPMG) pulse sequence, we use the recently-developed PROJECT sequence, which yields well-resolved, in-phase spectra. Taken together, these optimizations lead to high-quality T2-filtered 1H NMR spectra at spinning rates as low as 400 Hz, comparable both in resolution and signal intensity to the ones obtained conventionally at 4 kHz. These results demonstrate the performance of slow HR-MAS experiments for metabolic profiling of tissues, using well-established NMR pulse sequences. A metabolomics study was carried out with conventional and slow HR-MAS on liver samples from 3 groups of rats, submitted either to normal, fructose and fatty acids-supplemented or fructose, fatty acids and curcuma-supplemented diets. Both HR-MAS methods enable discrimination of the metabolic profiles of normally fed and supplemented rats. Interestingly, higher relative content of a selection of metabolites was found in samples analyzed with conventional HR-MAS, suggesting the release of some intracellular components into the solution surrounding the tissue and its tissue degradation at high spinning rate. These metabolites are detected in lower amounts when performing slow HR-MAS experiments. This suggests that these latter are expected to keep their original localization, leading to reliable metabolomics studies. Slow HR-MAS is shown to be a powerful non-destructive tool to perform metabolomics on fragile tissues.

POSTER 352

Utilizing LC-IMS-MS Measurements to Separate Lipid Isomers and Understand Their Role in Biochemical Processes

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Lipids play a vital role in many biochemical processes in biological and environmental systems. While there is great interest in understanding lipidomic processes, distinguishing lipids can be very difficult. Unlike the 20 amino acids in peptides, lipids are composed of very similar components such as double bonds and fatty acids arranged at distinct positions resulting in lipid isomers with vast functional differences. Coupling MS with separation techniques such as liquid chromatography and ion mobility spectrometry provides a basis for effectively distinguishing such isomers. In this study, we utilized LC-IMS-MS to analyze lipid isomers in complex extracts and gain knowledge about the specific roles that lipids have in the biochemical processes. Lipid standards and complex extracts were analyzed with a Waters HPLC and an Agilent 6560 IMS-QTOF MS platform using both positive and negative polarities to understand whether lipid classes and isomers were separable. Once injected into the nanoESI source, ions were passed through the inlet capillary, focused by a high pressure electrodynamic ion funnel, and accumulated in a lower pressure ion funnel trap before being injected into the IMS drift cell and refocused by a second ion funnel at the drift cell exit prior to QTOF MS detection. Initially, isomeric standards were evaluated manually and then added to LIQUID, the in-house lipid software, to increase its information content. LIQUID was then used for analysis of the complex lipid extracts. Over 500 different lipids representing phospholipids, saccharolipids, glycerolipids, sphingolipid sand fatty acyls were analyzed with the LC-IMS-MS platform. When only the LC dimension was analyzed, many of the classes eluted together even when long 100-min gradients were employed. Furthermore, the lipid subclasses (i.e. distinct phospholipids) were not separated under any gradient conditions evaluated. However, in the IMS dimension each lipid class had a distinct dependence of drift times to m/z (i.e. trendline) and even subclasses such as phosphatidylcholine and phosphatidylethanolamine could be distinguished. This information showed IMS to be a complementary technique to LC-MS and showed that utilizing all three dimensions (mass, drift time and elution time) was very important in characterizing and identifying unknown lipids. Lipid isomers consisting of enantiomers, cis/trans double bond orientations and positions, and sn-1/sn-2 chain differences were also analyzed with LC-IMS-MS since these isomers could not be distinguished in current LC-MS analyses. In the LC-IMS-MS studies, lipids with cis double bonds were found to be more compact than those with trans orientations, since the cis alignment positions the carbon chains in closer proximity. The location of the double bond also affected the lipid size with centrally located cis double bonds inducing smaller lipid structures than those with end positions. However, backbone locations for trans orientations could not be quantified, presumably due to the small structural changes to the lipid backbone. To illustrate this trend, four different bond locations and orientations were studied for the 18:1 fatty acid resulting in cis 11 Using LC-IMS-MS to separate isomeric lipid classes, enantiomers, cis/trans double bond orientations and positions, and sn-1/sn-2 chain differences.

POSTER 353

The Analysis of Bile Acids: Enhancement of Specificity Using Travelling Wave IMS-QTof Mass Spectrometry

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'Steroidomics' is the qualitative and quantitative study of steroid-type molecules found within the metabolome. Bile acids for example, are classified as acidic sterols that are synthesised mainly by the liver from cholesterol and aid digestion and fat solubilization. The presence of multiple species and iso-forms poses a great challenge for steroidomic research. Ion mobility-mass spectrometry (IM-MS) was combined with molecular modelling for the separation and configurational analysis of thirteen medically relevant bile acids. The usefulness of collision cross-section (CCS) information derived from IM measurements of relevant bile acids may be used to enhance specificity and augment steroidomic-type research and aid the diagnosis, prognosis and management of disease and will be discussed. ESI-MS

and MALDI-imaging were used to measure ion drift-times upon a hybrid IMS-Q-ToF mass spectrometer. In brief, the instrument comprises an IM separation device, a quadrupole and segmented collision cell prior to the TOF-MS. Ions are accumulated in the trap travelling-wave (T-Wave) and periodically released into the T-Wave IM where they separate according to their mobility. The IM separation is comprised of a travelling wave RF ion guide, which incorporates a repeating sequence of transient DC pulses to propel ions through the guide in the presence of nitrogen bath gas. Upon exiting the IM cell, ions can be selected with the quadrupole and undergo CID for structural elucidation information prior to detection with ToF-MS. We have investigated the use of IM as an analytical tool to aid bile acid identification. IM was used to measure the CCS values of 13 acids in negative mode using ESI. IM facilitates the calculation of the rotationally averaged CCS of an ion which can provide an insight into ionic configuration in the gas-phase. We have also used a distance geometry approach to generate molecular conformations of the bile acids, which were further used to measure the theoretical CCS with MOBCAL and PSA. The IM technique was shown to be able to distinguish between medically relevant isomeric deoxycholic, chenodeoxycholic, ursodeoxycholic and hyodeoxycholic acids. Deoxycholic and chenodeoxycholic acids have previously been detected in brain. These isomeric bile acids were rapidly distinguished by virtue of their differences in shape and could not have been separated by mass spectrometry alone. Both N2 and CO2 were explored as the mobility bath gas and the key differences in the mobility separation will be shown. We also provide evidence that these isomeric bile acids can be detected and IM separated off mouse brain tissue using MALDI Imaging-MS. Good correlation was observed between the experimental and theoretically derived CCS values. Negative ion bile acid CCS library generation using a travelling-wave based ion mobility mass spectrometer.

POSTER 354

A sensitive and robust LC-MS/MS method for the quantification of acylcarnitine species including low abundant short- and odd-chain acylcarnitine isomers

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Changes in plasma and urinary acylcarnitine profiles have been observed in complex diseases like obesity and diabetes. How acylcarnitines reach the plasma is still unknown but cellular release of acylcarnitines is thought to prevent intracellular accumulation of potentially toxic acyl-CoAs. Commonly, acylcarnitines are measured via flow injection-tandem mass spectrometry. This does not allow the separate quantification of isomer species, which is essential to identify for example their origin from fatty acid and amino acid breakdown. Although chromatographic separation of acylcarnitine isomers has been described, the abundance of various species is often too low for reliable quantification. We developed a sensitive HPLC-MS/MS method allowing separate quantification of acylcarnitines from different pathways, with a focus on amino acid-derived and odd-numbered acylcarnitine species. The HPLC-MS/MS method was developed using an Agilent 1260 Infinity Quaternary LC system coupled to an ABSciex QTRAP5500 MS system operating in positive electrospray ionization mode. Chromatographic separation was achieved on a Zorbax Eclipse EDB-C18 Column. Calibration lines were calculated using 13 deuterium-labeled internal acylcarnitine standards. Plasma and liver samples from wild-type C57BL6/J mice were used for method validation. For identification of individual peaks in tissues, reference compounds were synthetized and spiked into tissue samples. To improve chromatographic separation and enhance sensitivity, analytes were derivatized to the corresponding butyl esters. Tissues from diabetic mice were analyzed as a reference for known alterations in fatty acid and amino acid degradation. In total, 57 acylcarnitine species could be identified and quantified, including eight odd-numbered entities and most acylcarnitine species derived from breakdown of branched-chain amino acids, lysine and tryptophan. Calibration lines showed correlation coefficients of > 0.992. Precisions below 15% for high-abundant metabolites and below 20% for compounds of low abundance were reached and accuracy ranged between 82 and 108%. Analysis of samples from diabetic mice showed increased plasma and liver concentrations of intermediates of branched-chain amino acid degrading pathways and increased concentrations of odd-numbered acylcarnitine species, while even-numbered acylcarnitines remained unchanged. This new

method allows identification and quantification of novel acylcarnitines including odd-numbered species and “missing intermediates” in amino acid oxidation.

POSTER 355

Determination of Abscisic acid and Jasmonic acid Using UPLC-MS/MS Method

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Acidic plant hormones, such as abscisic acid (ABA) and jasmonic acid (JA), are a family of small molecul ar compounds with carboxyl groups, which regulate plant physiological processes, including seed germin ation, stem elongation, flowering and fruiting, and so forth. Many literatures have described the MS-based analysis of phytohormones. However, these methods were restricted to achieve satisfactory ionizat ion efficiency for acidic phytohormones. Herein, 3-bromoactonyltrimethyl
ammonium bromide (BTA) was used to derive abscisic acid and jasmonic acid by incorporating a positivel y charged quaternary ammonium group. The HPLC-
MS/MS analysis was performed using a 1290 Infinity LC coupled with a 6490 Triple Quad mass spectrom eter (Agilent, USA) using a Phenomenex silica column (250 mm×2 mm). Mobile phase A (MA) consisted of 0.3% formic acid in water, and mobile phase B (MB) consisted of 0.3% formic acid in acetonitrile. The fl ow rate was 200 μL min-
1, while the elution was under 75% of MB. The mass spectrometer parameters were as follows: ionization mode: ESI+; dry gas (N2) temperature and flow: 170 °C, 15 L min-
1; nebulizer: 35 psi; sheath gas (N2) temperature and flow: 400 °C, 12 L min-
1; capillary voltage: 3200 V; nozzle voltage: 0 V; fragmental voltage: 380 V.
In current study, we developed a highly sensitive method for the quantitative profiling of acidic phytohorm ones, such as abscisic acid (ABA) and jasmonic acid (JA). The tests for improving the pretreatment condit ions were conducted, and the methods for determination of ABA and JA in tobacco leaves based on ultra-performance liquid chromatography (UPLCMS/MS) were established. The limits of detection (LODs) of tar geted phytohormones was 0.082 ng/ml
and 0.027 ng/ml, which allowed the highly sensitive determination of low abundant acidic phytohormones with tiny amount plant sample. Good reproducibility was obtained by evaluating the intra-and inter-
day precisions with relative standard deviations (RSDs) less than 3.86% and 4.53%, respectively. Recove ries of the target analytes from spiked tobacco leave samples ranged from 96.4 to 110.8%. By employing the method developed here, we were able to simultaneously determine abscisic acid and jasmonic acid fr om only 25 mg of tobacco leave samples, which dramatically decreased the required sample amount for t he profiling of low abundant acidic phytohormones compared to previous reports. Taken together, the met hod provided a good solution for the highly sensitive and quantitative profiling of endogenous acidic phyto hormones. We developed a highly sensitive method for the quantitative determination of BTA-
derived acidic phytohormones by UPLC-MS/MS platform.

POSTER 357

A novel LC/MS method for the separation and quantification of dihydroxycholesterols and its application in RORgt receptor de-orphanization

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It is well known that mono-oxygenated products of cholesterol (OHC's), such as 25-OHC, 24(S)-OHC and 27-OHC, play important roles in lipid metabolism, immuno-suppression, and tumor cell growth. Recently, we discovered a dihydroxycholesterol (diOHC), 7α,25-diOHC, as the natural ligand for a G-protein-coupled receptor (GPCR) called EBI2, and its concentration gradient directs B-cell migration. It is often required to separate and quantify structurally very similar diOHC isomers (e.g., 7α,25-, 7β,25-, 7α,27-, 7β,27-) in native and in chemical derivatized forms to assess their distinct biological functions. Therefore,

we developed a novel LC/MS method for this purpose, which has, in addition to other projects, provided strong and unique supporting evidences in the identification of diOHC's as natural ligands of an important nuclear receptor, RORgt Synthesized oxysterols, animal plasma and tissues, as well as cell cultures were used in the experiments. Sample prep was facilitated with C18 solid-phase-extraction (SPE) as well as with liquid-liquid-extraction (LLE) of oxysterols. Separation methods were screened for native oxysterols using GC, reversed-phase HPLC (rpHPLC), normal-phase HPLC (npHPLC), and Supercritical Fluid Chromatography (SFC). HPLC methods were also optimized to semi-prep scales. Chemical derivatization of oxysterols with Girard's Reagent P (GP) was performed following protocols developed in W.J. Griffiths' lab. Separation and quantification of GPOSTER derivatized oxysterols were done with rpHPLC/scheduled Multiple Reaction Monitoring (sMRM) using deuterated internal (and surrogate) oxysterol standards. Extraction of oxysterols from biological samples was optimized for various animal plasma and tissues including brain, liver and spleen. An efficient GC/MS method was developed for oxysterol separation in native forms. Robust methods for semi-prep scale purification of native oxysterols with rpHPLC and npHPLC were also developed. For GPOSTER derivatized oxysterols, extensive rpHPLC protocols were tested and ACE C18-AR column was found to be most effective in separating most oxysterol derivatives down to baseline level (including 7α,25-, 7β,25-, 7α,27-, 7β,27-diOHC's). The rpHPLC/sMRM method was sensitive enough to quantify GPOSTER derivatized oxysterols in various biological matrices, while at the same time had the LC peaks fully resolved. The lower detection limit of the diOHC's with this method is about 10 pg, and the standard curves ranging from 100 pg/ml to 100 ng/ml (r = 0.98) were established. At 0.4 ng/ml the accuracies are less than 20% and intra-assay CV's less than 8%. RORgt is a nuclear receptor required for the generation of Interleukin-17 (IL-17) producing cells, and an important target for treating infections and autoimmune diseases; however, its endogenous ligands were not known. Through extensive biological experiments and applications of this rpHPLC/sMRM method to quantifying oxysterol levels in cells and tissues, researchers at Janssen R&D demonstrated that: In Cyp27a1 knock-out (KO) mice, the productions of 27-OHC and 7α/β,27-diOHC's were significantly lowered compared to that in the wild-type (WT); The same is true for the number of IL-17 producing cells. In addition, 7a/β,27-diOHC's but not 7α,25-OHC promoted IL-17 production by mouse and human Th17 cells in vitro. Armed with the robust and highly sensitive quantification of these low-abundant, structurally-similar and isobaric diOHC's, we concluded that certain oxysterols (esp. 7β,27-diOHC) are the endogenous ligands of RORgt. This result will greatly help the design of small molecular modulators of RORgt in drug discovery. A first in the field rpHPLC/sMRM method that separates structurally very similar yet functionally quite different dihydroxycholesterols after ESI/MS-sensitivity-enhanced GPOSTER derivatization.

POSTER 359

Comprehensive metabolic profiling of bile acids in human and mouse

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Bile acids (BAs) are a group of cholesterol-derived steroidal compounds with a wide range of cytotoxicity which is associated with pathophysiology. BAs regulate gene expression in liver for detoxification, lipid homeostasis, and energy expenditure. The determination of BAs in biological samples has the potential for broad applications in both basic and clinical research, including diagnosis, prognosis, and management of multiple human diseases. Recently, we developed a UPLC-MS/MS method for quantitation of 50 known bile acids in human and mouse blood (Han, et al. Anal Chem, 2015). In this work, we describe a combined UPLC/multiple-reaction monitoring (MRM)-MS and high-resolution MS method for the expanded metabolic profiling of more than 100 bile acids in a variety of human and mouse biological samples. Human blood/urine/feces and mouse blood/liver/bile/feces were used in this study. Chromatographic separations of various BAs were optimized by reversed-phase UPLC, and scheduled MRM was used for quantitative measurements on a triple-quadrupole instrument. The class-specific MRM transitions of known BAs were utilized for untargeted UPLC/MRM-MS detection of the potentially unknown BAs in different biological samples; the detection was complemented by UPLC/ultrahigh-resolution MS and UPLC/MS/MS on a LTQ-Orbitrap instrument, with the aid of carboxyl

grouPoster specific chemical derivatization using a hydrazine reagent. For UPLC/MRM-MS quantitation, blood and liver samples were spiked with 15 isotope-labelled BAs as internal standards followed by protein precipitation and phospholipid-depletion solid-phase extraction (PD-SPE). The concentrations of putative BAs were determined from the calibration curves of their known isomers. By using the class-specific MRM transitions of known BAs for untargeted LC/MRM-MS detection of the potentially unknown BAs in different types of human and mouse samples, a total of >100 BAs including at least 30 of their phase II metabolism products (i.e., sulfates and glucuronides) were detected. These compounds included at least 12 tetra-OH BAs which were mainly detected in mouse blood, liver and bile. BA sulfates were shown to have higher abundances than BA glucoronides in human urine, and sulfates of the unconjugated and glycine-conjugated mono- and di-OH BAs were the main forms of the phase II metabolites. In human and mouse fecal samples, the BA compositions were dominated by secondary BAs and their oxidation products (keto- and oxo-BAs) although most of these BAs were also detected in human blood, and mouse blood, liver, and bile samples, mostly at very low concentrations. Detection of potentially unknown bile acids were confirmed by the complementary techniques of UPLC/high-resolution MS and UPLC/MS/MS. Based on the preliminary untargeted detection, a scheduled MRM list was compiled to simultaneously quantitate 51 known BAs (30 unconjugated, 8 glyco-conjugated and 13 tauro-conjugated) and to determine the concentrations of the putative BAs by UPLC/MS/MS. To achieve better quantitation, PD-SPE was evaluated and found to be an efficient sample preparation procedure for quantitation of BAs in blood and liver, while reversed-phase SPE was superior for enrichment of BAs in human urine. The on-column analytical sensitivities of low femtomoles and linear ranges of 256 to 2056 fold in concentration (R2≥0.999) were observed. Quantitation accuracies ranged from 80% to 114% for most of the quantitated BAs, with intra-and inter-run CVs of ≤ 10.8%. In summary, this work provided a comprehensive analysis of BAs and their metabolites in human and mouse with some potentially new BAs quantified for the first time. Using UPLC/MS/MS, more than 100 bile acids and their metabolites in human and mouse were quantified for the first time.

POSTER 360

UPLC-MS/MS quantitation of aldehyde biomarkers of oxidative stress in human plasma using isotope-labeled 3-nitrophenylhydrazine for chemical derivatization

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Oxidative stress is a known feature of many health problems. It has been associated with predisposition to or manifestation of neurodegenerative diseases, cardiovascular disease, diabetes, cancer, and acute tissue injury. For some health conditions, oxidative damage has been proposed as a mechanism of pathogenesis. There is therefore a substantial interest in analytical methods for the sensitive and reliable measurement of various oxidative stress biomarkers. Low-molecular weight (LMW) aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal (HNE) result from peroxidation of lipids in biological systems. Here we report the development of a new ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC-MS/MS) method, in combination with a stable isotope-labeled chemical derivatization approach, for precise and accurate quantitation of LMW aldehyde biomarkers in human body fluids. 3-Nitrophenylhydrazine (3NPH) was used for chemical derivatization of 8 LMW aldehydes under acidic or weakly basic reaction conditions. A UPLC-quadrupole time-of-flight instrument was used to assess the products of the derivatizing reaction. The derivatization was optimized for reaction temperature and duration. Multiple-reaction monitoring (MRM) transitions for UPLC-MRM/MS quantitation were optimized via direct infusion of individual derivatives on an AB 4000 QTRAP triple-quadrupole mass spectrometer. A reversed-phase C-18 column was used for chromatographic separation with acetonitrile-water-formic acid as the mobile phase. Protein-bound aldehydes were released via hydrolysis with sodium hydroxide. The analytical method was validated to determine the analytical sensitivity, selectivity, precision and accuracy of the UPLC-MRM/MS method for assay of the aldehydes in human plasma. 3NPH was screened as a reagent to efficiently convert LMW aldehydes (MDA, acrolein, glyoxal, methylglyoxal, crotonaldehyde, methylbutryaldehyde, butanal, HNE) to their derivatives. To achieve complete, quantitative conversion, derivatization of MDA was carried out under strong acidic conditions

(0.1% TFA). The reaction followed a nucleophilic addition–elimination mechanism to form the stable derivative with a cyclic structure that favored positive-ion electrospray ionization (ESI)/MS. The reaction was optimal when performed at 75C for 60 minutes and no side reactions were observed. The use of a UPLC-MRM/MS method with an isocratic elution demonstrated on-column analytical sensitivities of 15 fmol for the limit of detection and 60 fmol for the lower limit of quantitation. To achieve precise and accurate measurements, 13C6-3NPH was used to produce a stable isotope-labeled analogue of the derivative, which was used as an internal standard to compensate for the unfavorable matrix effects of ESI. The method was applied to quantify both free and protein-bound MDA in human blood. Method validation found a wide linear (R2 ≥0.9996) range of >2000-fold concentrations, good intra- and inter-run CVs (0.999) and wide concentration ranges (>1000 fold) for UPLC-MRM/MS quantitation. We are currently undertaking further validation of this method for reliable quantitation of aldehydes in human plasma and urine, and its application in metabolomics studies. Experimental results will be presented. A new LC-MS/MS method with 12C/13C-labeled chemical derivatization is developed for quantifying aldehyde biomarkers of oxidative stress in human blood.

POSTER 361

Metabolomic analysis of aldoses, ketoses and carboxylic acids in wine by chemical derivatization - LC-MS/MS

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Wine has complex chemical compositions that are dependent on grape variety, growth environmental conditions, viticultural practices, yeast strains, as well as winemaking technologies and ageing processes. Metabolic profiling of wines has been the subject of many studies over the past decade. Among the diverse compounds, rapid identification and monitoring of concentration variations in low-molecular weight (LMW) sugars and carboxylates during the yeast (Saccharomyces cerevisiae) fermentation of grapes and in the finished wine products are particularly important because these compounds are not only important substrates or intermediates of fermentation but also influence the wine's organoleptic properties. We developed a chemical derivatization-LC-MS/MS method for the simultaneous determination of LMW sugars and carboxylates in wine. Fifteen mono- and di-saccharides were reacted individually with a derivatizating reagent, 3-nitrophenylhydrazine (3NPH), with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide as the condensing reagent and pyridine as the catalyst. The effects of reaction temperature, duration, and other parameters on the completeness of the derivatization were evaluated. The multiple-reaction monitoring (MRM) transitions of individual derivatives were optimized by direct infusion on an AB Sciex 4000 QTRAP triple-quadrupole mass spectrometer. Method validation was carried out to evaluate the analytical sensitivities, specificities, intra- and inter-run coefficients of variation, and the standard substance spike-in recoveries. Chromatographic separations were compared on different reversed-phase ultrahigh-performance (UP) LC columns for the simultaneous quantitation of the low-MW sugars and the 11 important fermentation-related carboxylates in wine. The optimization experiments indicated that 3NPH derivatization in 50 to 70% acetonitrile or methanol, and at 55 oC for 60 min, were the optimal conditions for complete conversion of 14 of the 15 sugars to their 3-nitrophenylhydrazones, which were suitable for analysis by negative-ion ESI/mass spectrometry (MS). A core-shell pentafluorophenyl UPLC column was found to be superior to other reversed-phase columns for separation of the sugar derivatives. With water:methanol:0.1% formic acid as the mobile phase for gradient elution and chromatographic optimization, good separations between the derivatives of different sugars -- including the structural isomers of disaccharides (lactose and maltose/cellobiose), four hexoses (glucose, galactose, fructose and mannose), two deoxy-hexoses (fucose and rhamnose) and four pentoses (ribose, xylose/arabinose, xylulose and ribulose) -- were achieved, while sucrose lacks a carbonyl group to react with 3NPH and did not interfere with the analysis of the other sugars. Method validation indicated that the on-column analytical sensitivities were at sub- to low picomolar levels. Using 13C-labeled internal standards, the 14 sugars showed good linearity over a range of 512 to 2056 fold in concentration. The intra- and inter-run CV% were below 11.5% (n=6) in all cases. The quantitation accuracy, as measured from the standard substance spike-in recoveries, was determined to be between 88.7% and 113.5% (n=6) for the quantifiable sugars in a wine. Finally, this proposed 3NPH derivatization-

LC/MRM-MS method for LMW sugars, together with a previously validated procedure for analysis of the central carbon metabolism carboxylates (Han, et al., Electrophoresis, 2013) using the same derivarization procedure, was used for simultaneous quantitation of the LMW sugars and carboxylates in three wines manufactured in the Okanagan valley region of British Columbia, Canada. Significant differences in the compositions of these LMW sugars and carboxylates were observed between the three wines tested. A new LC-MS/MS method was developed to simultaneously quantitate LMW aldoses, ketoses and carboxylates in wines for the first time.

POSTER 362

Microbial metabolomics in open microscale platforms

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Microbial secondary metabolism is an incredibly complex source of bio-active compounds that have important implications on human, animal, and plant health. In human disease, fungal secondary metabolites are produced in the host microenvironment with severe health consequences. The relevant metabolomics studies are limited, however, by current culture and metabolite isolation tools that do not allow the exploration the wide range of microenvironments. Here, we present a novel open microscale metabolomics technology that enables simple and integrated culture and isolation of microbial metabolites. The technology platform leverages a new class of microfluidic flows to create a biphasic interface over solid and liquid cultures of fungi and mammalian cells. These techniques enable accessible small molecule isolation and the integration of organotypic models. We engineered a microscale metabolomics platform that incorporates requirements of fungal culture and microfluidic flow of solvents in a simple embodiment. The technology enables multiple fold decreases in the volume of reagents required for culture and small molecule isolation (10 uL instead of 10 mL), allowing the use of rare sample and matrices that enables a passive biphasic flow of organic solvent over the aqueous or hydrogel culture area. Further, while traditional methods of fungal culture use flasks or petri dishes, which require large volumes of reagents, space in incubators or shakers, and processing time, the micrometabolomics platform fits tens of experimental conditions on a microscope sized device that can be processed in parallel using a simple pipette. We explored the impact of the choice of solvent as well as the potential to use high boiling point solvents in an experiment that compared the micrometabolomics platform to conventional culture and extraction tools. We extracted metabolites of Aspergillus nidulans using three different solvents, chloroform, 1-pentanol, and γ-caprolactone, which were chosen to cover a range of polarities. The micrometabolomics platform makes it feasible to use low-volatility solvents to explore segments of the metabolome that simply are not extracted when using more common solvents such as chloroform. Using the micrometabolomics platform, a large panel of microenvironments can be tested, including rare and expensive matrices that would traditionally be too costly. We performed an experiment to screen secondary metabolite production across a variety of environmental conditions, including three types of fungal inoculum, five types of fungal media, and two modes of culture. We chose different culture environments that represent chemical landscapes that can be found in the human lung, including the presence of blood cells and eicosanoids, which can cost hundreds of dollars per 100 ug. Preliminary data show that when A. fumigatus is grown on blood-agar compared to Glucose Minimal Media (GMM), there are evident changes in its global metabolite profile. MS-MS analysis of putatively identifies one of these over-produced compounds as a disease-relevant siderophore, TAF. Finally, we have performed microscale co-culture experiments involving neutrophils and germinating spores of A. fumigatus. Using lipidomic methods developed by Hammock and coworkers to identify and quantify oxylipins in microscale extracts we find that important immune mediators such as PGE2 and PGD2 are significantly reduced in the co-culture environment. These results suggest that immune cells interact with fungi to display an

altered eicosanoid response that could lead to opportunistic infections. This work demonstrates the enabling aspect of microscale systems to study host-relevant metabolomics with increased accessibility to large experimental spaces.

POSTER 363

Newly developed method of sampling suspension or adherent cell cultures for optimal metabolomics results

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The metabolome reflects the current biological state of an organism and the complex interactions between environment, genome, transcriptome and proteome. For suspension cultures, metabolomics pinpoints crucial optimization potential in feeding strategies, nutrient supply and process control for improved production quality or capacity. With adherent cells metabolomics is now becoming also an invaluable tool to study disease mechanisms, to discover drug targets, to observe resistance strategies and to investigate toxicities. For routine use of high quality metabolomics the sampling method is the key. This must be sufficiently robust, reproducible, simple, easily performable, and, most importantly, able to capture the physiological, non-perturbed intracellular metabolism. We developed such sampling methods and compared them to existing common protocols. Suspension cultures were fast filtrated, adherent cells were grown on LUMOX® dishes and harvested by membrane excision. The newly suggested sampling methods were thoroughly tested and compared to existing common sampling protocols. All collected samples were measured with our high-throughput, routine protocol (based on LC-MS/MS, GC-MS, UPLC–MS/MS) used for sample types such as plasma, blood, or urine, thereby leading to higher comparability of results between these different matrices. Multivariate (PCA) and univariate (ANOVA) statistical analysis was performed. Suspension cell cultures One can roughly divide sampling into either “quench first” (cold/solvent) or “separate first” (filtration/centrifugation). The obvious advantage of “quench first” is the earliest possible stop of metabolism avoiding degradation and interconversion, but necessitates validation for each new cell type to ensure leakage-free measurement. The obvious advantage of “separate first” is the leakage-free removal of extracellular contaminations and the importance of the contamination removal cannot be overemphasized. We concentrated on developing a fast filtration sampling method and identified the most critical factors for each step with bacterial (E. coli) and mammalian (CHO) cells. The new MxP® FastQuench method was designed to be also applicable to other, not yet tested cell types, with no or only minimal validation effort. Adherent cell cultures The two most common sampling procedures trypsinization and scraping were compared to the newly developed MxP® CellCollect technology for two breast cancer cell lines (MBA-MB-231 and MCF7) with routinely used extraction and metabolomics platforms. Metabolite levels differed tremendously revealing unavoidable and some avoidable issues with trypsinization and scraping risking to end up with non-physiological or misleading results in contrast to MxP® CellCollect. Trypsinization induces an energy depleted state while scraping was found to foremost insufficiently detach and dissolve lipidic metabolites yielding finally misleading results. Both developed methods deliver high quality results suitable to further standardization in in vitro cell metabolomics. Robust, reproducible, simple, easily performable, widely applicable cell harvesting methods able to capture the physiological, non-perturbed intracellular metabolism were developed.

POSTER 364

Novel high-throughput DNA quantification method for normalization of cell culture metabolomics data

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Metabolomics and its application to cell culture has proven itself as a potent tool in modern life sciences and is therefore becoming increasingly used. However, reliable data normalization methods still remain a challenge which has to be addressed, because erroneous normalization procedures will lead to flawed conclusions. Recently, a method using DNA for normalization was shown to be reliably suited (Silva et al. 2013). Nonetheless, the described method is not feasible for high-throughput analyses. We therefore developed and optimized a 96-well format, fluorescence-based method for DNA quantification with flexibility in regard to sample preparation and applied this method to cell culture samples. Cells (HEK293, Hep G2, HK-2, THLE-2, SGBS, 3T3-L1, COS-1, and Hepa1-6) were either harvested by scraping in methanolic extraction solvent or trypsinization and subsequent addition of the solvent, followed by homogenization. DNA in homogenates was quantified by the addition of a fluorescent dye and subsequent measurement of fluorescence intensity. We first optimized the dye concentration and sample volume. Afterwards, we recorded standard curves displaying the correlations of cell numbers with fluorescence intensity. In parallel, we quantified 188 metabolites by targeted metabolomics using the Biocrates AbsoluteIDQ™ p180 kit. Finally, we determined whether the fluorescent dye-based normalization method would be applicable, by evaluating the correlation between measured DNA concentrations and metabolite concentrations. Additionally, the impact of the applied harvesting methods was analyzed. We developed, optimized, and implemented a high-throughput feasible, robust, and cost-effective DNA quantification method, which is directly applicable to cell culture samples prepared for metabolomics. With this method, we obtained very good linear correlations ($R2 > 0.9$ for all tested cell lines) between the measured fluorescent signal and the deployed cell numbers (1E4 to 1E6 cells). Additionally, we proved that DNA quantification is a suitable tool for metabolomics data normalization, by harvesting different cell numbers of THLE-2, Hep G2, SGBS, and HK-2 cells within the range of 7.5E4 to 2.5E6 by trypsinization and scraping and performing targeted metabolomics. The results show, that the cell number correlates in a linear manner with the measured metabolite concentrations. This DNA quantification method is flexible and robust in regard to cell line choice, culturing conditions and harvesting procedures, and is now frequently applied by us in cell culture based projects using targeted (Biocrates AbsoluteIDQ™ and LC-MS/MS based drug quantification) and non-targeted metabolomics (Metabolon Inc. platform). Further, by using this DNA quantification method, we compared two distinct cell harvesting methods (scraping vs. trypsinization), without risking the introduction of a technical bias. The harvesting method impacts the recovery of metabolites in a metabolite class and cell line dependent manner. Fast, robust, and cost-effective high-throughput DNA quantification method, excellently suited for normalization of cell culture metabolomics data.

POSTER 365

Optimizing the harvesting and extraction method of adherently growing cells for non-targeted metabolomics analysis

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Cultured cells represent excellent models for research on all aspects of cell biology, human diseases, and drug development. As such, they are increasingly used for targeted and non-targeted metabolomics studies. Non-targeted metabolomics aims at the unbiased, simultaneous identification and relative quantification of as many metabolites as possible. When cell culture samples are analyzed, the procedure

how cultured cells are harvested and metabolites are extracted, has a large impact on the quality of the results. Addressing this challenge, we tested sampling methods for non-targeted metabolomics analysis, in view of i) detecting as many metabolites as possible, ii) enabling monitoring of the extraction efficiency, iii) making data normalization possible. Adherent cell lines (Hep G2 and HEK293), grown in monolayers, were used for the optimization of the cell harvesting and metabolite extraction procedure. We evaluated two metabolite extraction methods: in-well extraction and scraping. For in-well extraction, we applied the extraction solvent to the monolayer, incubated for 5 min, and collected the solvent. With the scraping method, the cells were scraped in extraction solvent, collected together with the solvent, and homogenized. For monitoring the extraction efficiency we added four standard chemicals to the extraction solvent. We also analyzed the influence of the methanol content of the extraction solvent. Detection and relative quantification of the metabolites was done using UHPLC-MS/MS. The MS2 data was matched to Metabolon's database library for metabolite identification. Comparing the two different sampling methods, we observed that scraping the cellular monolayer in methanolic extraction solvent was superior to in-well metabolite extraction in regard to the number of detected metabolites as well as the observed CVs. Furthermore, the scraping procedure allowed for the measurement of the DNA amount in the same sample. This was not possible with the in-well extraction procedure in which only solvent is collected. Analysis of the DNA content offers the possibility for data normalization (second POSTER on this conference). Moreover, we could show that the addition of four standard compounds to the extraction solvent facilitated a tight monitoring of the extraction efficiency, and we recommend to regularly implement this step for quality control. The extraction with three methanol concentrations (60%, 80%, and 100% methanol in water) yielded consistent and highly reproducible results. However, the comparison of these different extraction solvent compositions showed that the methanol content has a distinct, metabolite class dependent influence on the metabolite levels. This offers the flexibility to adapt metabolite extraction conditions according to individual study designs/requests. Fast and flexible harvesting and extraction method for non-targeted cell culture metabolomics.

POSTER 366

Vienna Metabolomics Center

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Metabolomics, the unbiased profiling of all small molecules in biological samples, has developed into a core technology for functional genomics, biobased economy and personalized medicine in the last decade. Recently, ecological metabolomics has emerged in ecosystem research and metagenomic studies. The quality of state-of-the-art metabolomics technology depends on the quality of the preparation workflows, the quality of metabolite libraries and workflows for structural elucidation of novel compounds. Because of the diversity of the metabolomes from organisms in functional and biomedical studies as well as the complexity of exo-metabolomes in environmental samples from marine, fresh-water and terrestrial ecosystems, an interdisciplinary approach is necessary to uncover novel aspects. Internationally recognized labs at the U Vienna distributed over three faculties for Chemistry, Geosciences and Life Sciences consolidate their specific and complementary research platforms and application fields to build a Vienna Metabolomics Center. The following labs are participating in this effort. Faculty of Life Sciences: Wolfram Weckwerth (Coordinator), Dept. Ecogenomics and Systems Biology; Christa Schleper, Dept. Ecogenomics and Systems Biology; Verena Dirsch, Dept. Pharmacognosy Thierry Langer, Dept. Pharmaceutical Chemistry; Walter Jäger, Dept. Pharmaceutical Chemistry; Jürgen König, Dept. Nutritional Sciences; Karl-Heinz Wagner, Dept. Nutritional Sciences; Gerhard J. Herndl, Dept. Limnology and Bio-Oceanography. Faculty of Chemistry: Gunda Köllensperger, Institute for Analytical Chemistry; Christopher Gerner, Institute for Analytical Chemistry; Veronika Somoza, Dept. Nutritional and Physiological Chemistry; Doris Marko, Dept. Food Chemistry and Toxicology. Faculty of Earth Sciences, Geography and Astronomy: Daniel Birgel and Jörn Peckmann, Dept. Geodynamics and Sedimentology, Stephan Kraemer, Dept. Environmental Geosciences. The faculty of the Vienna Metabolomics Center will cover a broad range of technologies from drug development, nutritional sciences, microbial and plant biotechnology, biomedical approaches up to ecosystems research and environmental sciences. Experimental output from all platforms is functionally integrated in a multidisciplinary approach

comprising methods from the fields of bioinformatics and mathematical modelling. A detailed description of the faculty will be presented. Further information is available at http://metabolomics.univie.ac.at/ A platform for multidisciplinary metabolomics approaches and functional data integration is presented.

POSTER 367

Microbial metabolomics: the importance of metabolome sample preparation

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Saccharomyces cerevisiae and Clostridium acetobutylicum, important strains in industrial fermentations, have received increased attention for producing ethanol, butanol, and industrial biochemicals. To obtain high productivity in producing those chemicals, vigorous manipulation of metabolism of S. cerevisiae and C. acetobutylicum is essential, and metabolic engineering is the key tool. For further improvement of metabolically engineered strains, the capability of metabolomics such as elucidating cell metabolism of engineered cell can be importantly used. To get accurate and reliable metabolomics data, well-established and customized metabolome sample preparation depending on microbial classes is necessary. In this study, we have evaluated and improved metabolome sampling methods for S. cerevisiae and C. acetobutylicum. We systemically evaluated and optimized metabolome sampling method such as currently used quenching method and extraction solvents for the metabolomics of a yeast, S. cerevisiae and a Gram-positive strict anaerobic bacterium, C. acetobutylicum, using gas chromatography/time-of-flight mass spectrometry. In yeast, a serious loss of intracellular metabolites was observed due to cell leakage when cold methanol quenching was used. Therefore, the fast filtration method was recommended and developed as an alternative to the cold methanol quenching. Also, acetonitrile/water mixture (1:1, v/v) at −20°C was the superior solvent for extracting metabolome of filtered S. cerevisiae. In C. acetobutylicum, regardless of its strict anaerobic nature, atmospheric processing did not significantly differ from anaerobic processing in quantity and quality of metabolomic data. For extraction of metabolome, pure methanol at −20 °C was selected as the best solvent for the strain. The approach used in this study could be used as a standard protocol for developing customized metabolome sample preparation.

POSTER 368

Novel NMR- and MS-Methodologies for the Analysis of Key Metabolites in their Phosphorylated and Non-Phosphorylated Forms

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Cellular life and central metabolism, e.g. glycolytic pathways and their regulation, depend on phosphorylated and dephosphorylated metabolites. As phosphorus occurs in many metabolite structures ranging from very stable compounds with a half-life of years to highly reactive intermediates with a half-life of minutes, the preparation of stable metabolite standards is an important prerequisite for quantitative analytical assays. Detailed molecular analysis has been key for the elucidation of central biochemical pathways and continues to be of major importance today. As metabolite chirality is relevant for cell metabolism in health and disease, the analysis of metabolite enantiomers is opening new areas of research and new applications, but going beyond the current limits of technologies requires the synthesis and analysis of metabolite enantiomers. Quantitative NMR and MS-methodologies have been developed to analyze and identify the new metabolite standards for the first time. As a prerequisite new generally applicable methodologies for the preparation of metabolite standards which so far have not been synthesized in pure form had to be developed. Quantitative 31POSTER NMR has been used for stability analyses of phosphorylated metabolites, for their formation in continuous and direct kinetic analyses and to characterize the enantioselectivity of kinases. This has been of relevance for reducing the number of reaction steps in the direct biocatalytic asymmetric synthesis of metabolite enantiomers. Novel high-performance LC- and CE-separation methods using charged aerosol detection or MS have enabled the analysis of closely related metabolite isomers as well as metabolite enantiomers. Stability windows have been determined for a range of phosphorylated metabolites. A remarkable result was that compounds with a high phosphorylation potential like phosphoenolpyruvic acids or adenosine 5'-triphosphate are

stable over a very broad pH range, whereas for example the central metabolite D-glyceraldehyde 3-phosphate or the toxic L-glyceraldehyde 3-phosphate are only stable under acidic conditions. Direct separation methodologies for metabolite enantiomers in their phosphorylated as well as in their non-phosphorylated forms have been achieved for the first time. The enantiomeric purities of aldehyde metabolites in their phosphorylated forms have been determined by HPLC after derivatization using chiral columns. The absolute configurations have been conformed by oxidation to the corresponding carboxylic acid enantiomer and by comparison of its measured optical rotation with literature values. There is a lot o be discovered and a bright future in these novel metabolomics technologies for the analysis of key metabolites in their phosphorylated and non-phosphorylated forms. Novel methodologies have been developed for analysing key metabolites in their phosphorylated and non-phosphorylated forms as well as their enantiomers.

POSTER 369

Enhancing versatility in LC-MS based metabolomics by combining targeted and non-targeted approaches

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With the advent of LC-MS based non-targeted metabolomics, large datasets containing information for a multitude of intracellular metabolites and pathways have become readily accessible. However, effective strategies for filtering and extraction of information relevant to the biological system to be studied are yet poorly defined. As such, critical information relating to significant metabolic changes may be lost amongst the overwhelming amount of information within metabolic fingerprints. The implementation of hypotheses and prior knowledge such as the well-known network of intracellular metabolic reactions could provide the next step towards efficient data evaluation and interpretation. We suggest that a combination of targeted and non-targeted approaches implemented within broad-coverage analytical platforms is a powerful tool for hypothesis generation beyond metabolic fingerprinting. We address the primary metabolome with a state-of-the-art analytical toolset including sample preparation and internal standardization with stable isotope-labeled cell extracts. The availability of several LC-MS based analytical platforms involving single- and two-dimensional liquid chromatography coupled to tandem mass and high-resolution mass spectrometry enables the tailoring of the analytical approaches to the analysis of various biological model systems. Data evaluation and statistical evaluation are performed within the Agilent MassHunter and MassProfiler Professional workflow. The presented case studies show how even small adjustments to the analytical strategy allow pinpointing of metabolic pathways without sacrificing the richness of non-targeted data sets. For instance, the presented two-dimensional heart-cutting approach combining reversed-phase and porous graphitized carbon liquid chromatography extends the metabolome coverage of conventional LC-MS based methods to biologically important, but analytically challenging compound groups such as sugar phosphates. Furthermore, a critical evaluation of typical data processing strategies revealed bottlenecks in the assessment of relative changes and significance testing. A novel on-line combination of orthogonal separation techniques increases the versatility in non-targeted metabolomics.

POSTER 370

Utilization of complementary ionization techniques, enhanced chromatographic resolution and mass spectral resolving power for characterization of yeast metabolites

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Gas chromatography - time of flight mass spectrometry, a “Gold Standard” for metabolomic studies, has been successfully implemented for the study of blood, plasma, urine, and plant materials. The next generation instrument for metabolomic exploration combines the power of GC×GC with high resolution time-of-flight mass spectrometry (GC×GC-HRT). GC×GC-HRT greatly exceeds LC-based chromatographic capabilities and was successfully utilized for identification of acids, diacids, amino acids,

sugars, fatty acids, nucleosides, nucleotides and phosphorylated metabolites in yeast. Yeast samples were spiked with isotopically labeled standards, extracted with 1:1 methanol/chloroform and filtered into 2mL GC vials. Sample components were derivatized using a short, two-step procedure and then analyzed by GC×GC-HRT using complementary EI and CI techniques. Compounds were separated using an Rxi-5MS column in the 1st dimension and Rxi-17 Sil MS in the 2nd dimension and detected with the HRT operating at a resolving power of 25,000 (m/z =218.98508). System performance was monitored using internal standards (e.g., octafluoronaphthalene and fatty acid methyl esters). Confident characterization of metabolites was accomplished through spectral deconvolution and database searches combined with accurate mass formula generation (<1ppm). This study resulted in the confident identification of hundreds of compounds in yeast extract samples. Compound characterization was facilitated through effective peak deconvolution which was critical for identification of coeluting labeled and native components. This was clearly evident from the ability of software to separate and provide deconvoluted spectra for coeluting D5, 13C11 and native tryptophan (3TMS). Native compounds were matched to spectra in large, well-established databases (NIST, Wiley, etc.) and accurate mass molecular, adduct and fragment ions were leveraged to confirm the identity of metabolites through formula determination. For example, the average mass accuracy and spectral similarity values for a representative set of derivatized amino acids (Glycine, serine, methionine, 5-oxo-proline, aspartic acid, ornithine, phenylalanine, glutamic acid, asparagine, lysine, tyrosine and tryptophan) were 0.72 ppm and 916/1000 respectively. Enhanced chromatographic and mass spectral resolution were particularly useful for increasing the number of confidently identified compounds. For example, in a one dimensional chromatographic run 2,3,4-trihydroxypentanoic acid (4TMS) coelutes with several compounds including asparagine (3TMS). The addition of an extra dimension of chromatographic resolution resulted in improvement of the spectral similarity score for trihydroxypentanoic acid from 617 in the one dimension data (GC-HRT) to 923/1000 in the two dimensional data (GC×GC-HRT). The combination of high resolution chromatography and high resolution time-of-flight mass spectrometry is a powerful technique for the characterization of metabolites. Unprecedented methodology combining GC×GC for enhanced chromatographic resolution with high resolution time-of-flight mass spectrometry for rapid and confident compound identification.

POSTER 371

Boosting metabolite characterization using ion mobility based high-resolution MSn

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Metabolite characterization has been regarded as the Achilles heel of metabolomics due to the great analytical challenges. In most metabolomics experiments, only a few hundreds (typically 150~250) of metabolites could be unequivocally or tentatively identified by comparing the MS2 and retention time of detected ions with those of reference compounds or MS2 libraries. Fast and confident determination of the identity of metabolites was still difficult even using high-resolution mass spectrometers. Here, we demonstrated ion mobility based high-resolution MSn allows easy spectrum interpretation and high confidence in metabolome-wide structural elucidation of human urine metabolites. An ultra-performance liquid chromatography/three traveling-wave ion mobility quadrupole time-of-flight mass spectrometry (UPLC/TW-IM-QTOF) was used for the characterization of human urinary metabolome. We first employed the ion-mobility MSE mode to simultaneously obtain MS1 and MS2 of all dateable precursor ions and discovered chromatographic unresolved isomers. Subsequently, we obtained MS1 and MS2 of detected metabolites as well as their drift time by flexibly varying the collision energy in the first and third ion guides of the Triwave. The IM-MS data was processed using DriftScope 2.1 (Waters Corp., Manchester, UK) to extract the drift time. By parallel or staggered dissociation the first and third ion guides, we obtained large-scale MS1 and MS2 TOF mass spectra of human urinary metabolites. This high resolution MSn repository could expedite SWATH, all-ion-fragmentation, and MSE based data-independent metabolite quantitation by targeted ion extraction. With the benefit from ion mobility separation, all product ions sharing the exact same m/z (mass error < 10ppm) and drift time were confidently assigned as the same structural building blocks of their precursors which could be classified into the same subgroup. Once a metabolite in this subgroup was identified, the corresponding diagnostic building blocks were also identified. These diagnostic building blocks facilitated de novo structure elucidation and narrowed the

database hits of other unknown metabolites. Further structure elucidation was facilitated by MS3 and neutral loss based network analysis. With the above strategies, we achieved the most comprehensive profiling of human urine with more than 500 metabolites being on-line characterized. Ion mobility based high-resolution MSn characterized more than 500 human urinary metabolites

POSTER 373

Expanding the Coverage of Metabolites Using Multiple Liquid Chromatography Separations

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Mass spectrometry-based metabolomics is the comprehensive study of naturally occurring small molecules collectively known as the metabolome [1]. Given the vast chemical and physical properties of endogenous metabolites and their wide concentration range, to reduce the sample complexity using liquid chromatography separation as the frontend is widely used for expanding the coverage in global metabolomics studies. While reversed phase RPLC (C18 based) being the most widely applied method in metabolomics study, a few alternatives like hydrophilic interaction chromatography (HILIC) and mix-mode have also obtained increasing attention recently owe to the improved retention for polar metabolites. However, a comprehensive comparison of these methods is still lacking, especially using a relative large panel of compounds representing the vast diversity of real complexity. Metabolite standards were weighed and dissolved in MeOH/H2O at the concentration of 0.5mg/mL. All LC methods were conducted on a Thermo Scientific Dionex Ultimate 3000 HPG system. For RP, a Thermo Scientific Hypersil Gold C18 column (150x2.1mm, 1.9um) was used, with a run time of 15 min. For HILIC, a Merck SeQuant® ZIC-pHILIC (150 x 2.1 mm, 5 um) was used, run time 27 min. For mix-mode, a Thermo Scientific Acclaim Trinity P1 (100 x 2.1 mm, 3 um) was used, run time 22 min. Thermo ScientificTM Q ExactiveTM mass spectrometer was used for the detection. Full MS was acquired using positive/negative switching mode at resolution 70,000. Targeted MS/MS was acquired separately to confirm the identities at resolution 35,000. The metabolites generally divided themselves into 3 categories with RPLC separation. (A) Nonpolar, showing good retention with nice peak, e.g., 5-Hydroxyindole acetate, (B) Polar, showing early elution but still single fine peak, e.g. γ-Aminobutyric acid, and (C) Difficult ones (including both polar and nonpolar), showing all kinds of bad chromatographic peaks, e.g., broadening, splitting (e.g., Tryptamine), fronting, tailing, or low response. 125 metabolites fall into category A, meaning they are well retained and have good peak shape on RPLC, with the retention time spanning 1.2 to 8.3 min along the total 15 min (corresponding to retention factor (k) =0.50-10.9), while 77 out of 125 show good retention on HILIC with k= 0.4-9.2 and 87 show good retention on P1 with k=0.1-32.4. 92 metabolites fall into category B, meaning they demonstrate good peaks on RPLC but no retention (less than 0.8 min). Among these compounds, 88 show very good retention on HILIC with k=1.4-13.1 and 66 show good peaks on mix-mode but the majority elute in void volume like in RP. There are 80 metabolites in Category C, showing bad peaks on RP, but 41 of them are well retained by HILIC and 45 by P1 (k>0.5). Overall, RPLC detected 217 ((A)125 + (B)92+(C)0) good-shape peaks out of 300 total metabolites, then 206 ((A)77+(B)88+(C)41) on HILIC and 198 ((A)87+(B)66+(C)45) on mix-mode. If excluding the void volume peaks (less than 0.8 min), which might produce false positives in quantitative analysis because of the co-elution of a large amount chemical similar species, in this case, HILIC gives the best number 206 followed by RP (145) and then mix-mode (135). RP and mix-mode are complementary to HILIC method in global metabolomics study. Mix-mode approach shows promising separation for specific compounds that are challenging to RP, which needs further investigation. A comprehensive comparison of three liquid chromatography methodologies with 300 endogenous metabolite standards

POSTER 374

Pathogen biofilm analysis using novel metabolomics technologies

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Pathogen biofilms are currently held to be responsible for ~80% of infections, and are a growing problem in the fields of hospital acquired infections (HAI) and orthopaedic surgery. Novel methods must be developed to modulate biofilm growth. Metabolomics is intended to analyse the complete small molecule component of a biological system. Analytically, the diversity present in these compounds creates huge problems. Standard chromatographic methodologies in metabolomics use individual columns chemistries to obtain separations that are by nature biased towards certain classes of these compounds. The aim of this study was to develop a method that could simultaneously retain and separate small polar and large nonpolar metabolites in one chromatographic run. In this study we implement a separation that effectively retains both polar and nonpolar compounds by serially coupling a reversed-phase liquid chromatography (RPLC) column to a hydrophilic interaction liquid chromatography (HILIC) column via a T-piece. Two independent pumps were incorporated into the system to allow independent gradient control of the two columns. A 1 x 100 mm C18 and a 4.6 x 150 mm zwitterionic HILIC were run at a flow rate of 65 µL/min and 300 µL/min, respectively, with ESI-FTMS for analyte detection. The high dilution between the columns, achieved by the difference in flow rates, allowed each of the methods to be optimised independently to suit sample requirements. We demonstrate the retention and separation of both polar and nonpolar standards and numerous polar and nonpolar metabolites extracted from beer. The samples were initially injected onto the RPLC column in high aqueous conditions. The nonpolar metabolites were retained by the RPLC column, while the polar metabolites that were not retained were introduced into the HILIC column in high organic conditions, where they were retained and separated. In the application of the technique to a complex sample (beer) we demonstrate the separation of hydrophobic compounds such as alpha-acids as well as polar compounds such as TCA cycle intermediates and amino acids. The method was then applied to the analysis of pathogen biofilm cultures with the goal of identifying pathways controlling their coexistence as a means of understanding their role in clinical infection. The results obtained from this study validate this simple yet powerful global metabolomics approach. The first time this method has been used to separate and retain nonpolar and polar compounds within a complex sample.

POSTER 375

Optimized LC-MS Platform for Large-scale Untargeted Metabolic Profiling of Human Urine and Plasma

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Profiling of body fluids is crucial for monitoring and discovering metabolic markers of health and disease and for providing insights into human physiology. Since human urine and plasma each contain an extreme diversity of metabolites, a single liquid chromatographic system when coupled to mass spectrometry (MS) is not sufficient to achieve reasonable metabolome coverage. Hydrophilic interaction liquid chromatography (HILIC) offers complementary information to reverse-phase liquid chromatography (RPLC) by retaining polar metabolites. However, most untargeted metabolomic studies are performed using RPLC-MS alone and often with suboptimal conditions. This observation motivated us to find an optimal chromatographic solution involving orthogonal HILIC- and RPLC-MS that will enable the optimal reproducible coverage of human urine and plasma metabolites. To achieve optimal profiling of human urine and plasma, we systematically investigated the performance of five popular HILIC columns with different chemistries operated at three different pH (acidic, neutral, basic) and five C18-silica RPLC columns using standard metabolites and biological samples. Standard mixtures, urine and plasma samples were analyzed using an Agilent 1260 Infinity HPLC system coupled to an Agilent 6538 UHD Q-TOF MS. To facilitate the comparison of the different chromatographic conditions, a qualitative scoring system that assigns a score to each metabolite was created. The score was calculated based on the

retention time, peak shape, and MS signal. The zwitterionic column ZIC-HILIC operated at neutral pH 6.9 provided optimal performances on a set of 137 hydrophilic metabolites representative of the chemical diversity that populates urine and plasma. Interestingly, the RPLC columns Hypersil GOLD and Zorbax SB aq were best suited for the metabolic profiling of urine and plasma, respectively. Combining the optimized HILIC- and RPLC-MS greatly expanded the metabolome coverage compared to RPLC alone. About 44% and 108% new metabolic features were detected enabling to monitor over 16,000 and 9,000 distinct features in urine and plasma, respectively. Importantly, we found that the ZIC-HILIC column was adequately equilibrated and conditioned after the injection of 12 biological matrixes. This resulted in excellent intra-batch (average CV of 11.4% ± 0.9%) and good long-term inter-batch (40 days) peak area reproducibility (average CV < 22%) similar to RPLC-MS approaches. Altogether, the combined LC-MS approaches improve the comprehensiveness of global metabolic profiling of body fluids and thus are valuable for monitoring and discovering metabolic changes associated with health and disease in clinical research studies. Inter-batch normalization is a significant hurdle of large-scale untargeted metabolomic studies. MS signal drifts with time and cannot be easily corrected by using internal standards because it is nonlinear and is metabolite specific. Some data-driven normalization methods, among which sum normalization is the most popular, are commonly used. We have injected the same urine sample onto ZIC-HILIC column in nine different batches, the last one acquired after 215 days. Sum normalization couldn't correct for the MS signal drift between the first and the last batch exhibiting an average CV of 38%. This variability could be efficiently minimized using LOESS normalization with an average CV of 20%. This unique dataset shows that LOESS normalization is very efficient to correct MS signal drift with time and enables accurate large-scale untargeted metabolomic studies. This is the first study describing an optimal analytical platform for large-scale untargeted metabolic profiling of human urine and plasma.

POSTER 376

Quantitative Mass Spectrometry to Study the Effect of Sample Handling and Storage on the Human Milk Bioactive Lipid Metabolome

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Human breast milk is the first nutritional and functional food for the new born baby. It is a potential source of bioactive lipids such as endocannabinoids and oxylipins, which may protect against infection, inflammation, and spur immune system and organ development. But there is still a limited amount of studies given serious attention to sophisticated analytical techniques for bioactive lipid profiling. Furthermore, there is little information about specific storage conditions and handling protocols that can minimize error and variability. It's an urgent topic to detect the bioactive lipid metabolome efficiently and to examine the storage and handling conditions that influence the quality of bioactive lipid compositional analysis. The effects of extraction state (fresh or frozen), storage temperature (4°C, -20°C or -80°C) and different storage duration (1 day, 1 week or 3 months) at different condition prior to solid-phase-extraction (SPE) of the bioactive lipid metabolome were investigated. We developed a method to simultaneously detect endocannabinoids and oxylipins metabolomes by ultra-performance liquid chromatography coupled to tandem mass spectrometry (UPLC-MS/MS) techniques on an Agilent 6490 instrument. Two-way ANOVA with turkey's multiple comparison test was used to detect significance between different condition from fresh samples for each compound analyzed, and t-test was used to compare the levels. Thirteen endocannabinoid standards and thirty-seven oxylipin standards were quantified with targeted metabolomic methods using UPLC-MS/MS. All of the endocannabinoids were detected with levels ranging from 0.002 to 111 nM. NAGly was only found in the fresh milk samples with levels ranging from 0.002 to 0.006 nM. Furthermore, 2-AG was found in the highest levels followed by AEA, SEA and PEA in fresh milk. Most of the endocannabinoids, such as 2-AG and SEA increased dramatically when they were kept at 4°C. They increased even more when they were kept at 4°C for longer time. Some endocannabinoids such as PEA, OEA and LEA were not so sensitive to the storage condition when analyzed within one day of collection, but increased significantly after 1 week. Thirty of the oxylipins, representing products from all three degradation pathways, cyclooxygenase, lipoxygenase and cytochrome P450, were detected with levels ranging from 0.012 to 1242nM. The resolvin D-family

precursor 17(R)-HDoHE was found in the highest levels followed by 8(9)-EET, 13-HODE, 9(S)-HODE, and 12,13-DiHOME in fresh milk. Like endocannabinoids, most oxylipins changed dramatically when they were stored at 4°C. 8,9-DHET, 9,10-DiHOME, PGE2 and the HETE subfamily members, such as 5-HETE, 8-HETE, 9-HETE and 20-HETE showed markedly increased levels after storage at -20°C for 3 months. The findings of this study suggest that except for analysis of fresh samples or samples within one day of collection, preservation at -20°C for no more than one week is necessary and for long term storage, -80°C is a requirement. Storage at 4°C prior to SPE introduces significant variability in most endocannabinoids and oxylipin levels. To present a protocol for investigating bioactive lipid metabolome in human milk, which can be applied in related physiological studies.

POSTER 377

Evaluation of heat stabilization for the conservation of biological samples as an approach for metabolomics: a 1H-NMR/LC-MS metabolite profiling study

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In metabolomics, sample preservation is key for the relevance of the analytical results. Indeed, mainly because of the presence of functional enzymes, metabolite content in biological samples changes rapidly and it is necessary to completely block all residual activity. Today, the most admitted and validated procedure consists in snap freezing and long term storage at -80°C. However, freezing induces a non-permanent blockade of some enzymes and changes may occur during sample preparation/analysis. Heat stabilization, a technique developed for the preservation of biological samples, induces permanent enzyme inactivation. Thus, heat stabilization has the potential to be an efficient method for metabolites preservation in metabolomics studies and this works aims at evaluating it by a 1H-NMR/LC-MS integrated metabolite profiling. The study is performed on mouse livers from 30 healthy mice, randomized in 5 groups of 6 animals designed to compare snap freezing at -80°C to 3 heat stabilization conditions (freezing just after heat stabilization, after 1 hour and after 3 hours) and to a negative control maintaining samples at room temperature during 10 minutes before freezing. For each mouse, livers are split in 2 equal samples which are extracted separately for polar and apolar compounds and analyzed using 1H-NMR, on a 600 MHz spectrometer with cryoprobe, and LC-MS, on a Q-Exactive Orbitrap and a Maxis HD Q-Tof, for integrated metabolite profiling. Heat stabilization procedure is performed on a Stabilizor system from the Denator Company. All liver samples have been collected and stored following the protocol. Heat stabilization procedure, which is automatized with the Stabilizor system, has been monitored and shows a good temporal repeatability and short delay of processing (20sec.) which allows us to have homogeneous samples for the analytical part of the study. Extraction protocol for polar and apolar samples have been defined and validated on residual liver samples and show a good phase separation and clean extracted solution enabling direct LC-MS injection in parallel of NMR. LC-MS methods are currently finalized and in the validation step. First data in NMR on polar extracts show clean and informative spectra with a good representativeness of different chemical families, such as amino acids, ketone bodies or alcohol. These preliminary results suggest that our protocols will allow a comprehensive metabolite profiling of the biological samples and thus will give us the opportunity to thoroughly evaluate the heat stabilization as a preservation procedure for metabolomics. New biological sample preservation procedure based on heat stabilization for metabolomics and integrated NMR/LC-MS metabolite profiling.

POSTER 378

Comparing metabolomics data from four identical GC-TOF MS platforms to evaluate instrument drift and batch effects in large-scale studies

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GC-MS analysis of large sample sets is common in metabolomics. Such analyses require special attention from sample preparation to data processing as batch effects, particularly in very large studies, become more problematic over time. Several quality control measures can be taken to minimize batch effects, moderate instrument drift, and produce more consistent data for better statistical approaches. We analyzed human citrate- and EDTA-plasma samples on four identically configured GC-TOF MS platforms from the same manufacturer, to determine data variation produced within and between instruments for most effective use of instrument and processing time. Commercially available human blood plasma was purchased from Rockland, Inc. and BioreclamationIVT. One technician performed all extractions in one day, then dried and stored them at -80°C until analysis. 30µL was extracted in acetonitrile:isopropanol:water (3:3:2 v/v/v), then 50% acetonitrile (v/v), and divided into equal aliquots for simultaneous analysis on all instruments, totaling 30 samples per instrument every three months for one year. Following MSTFA-derivatization, 0.5µL sample was injected on Agilent 6890/7890 GC with RTX5-MS column (30m x 0.25mmID x 0.25µm) at 1mL/min constant helium flowrate, coupled to Leco-PegasusHT TOF. Temperatures were: 50°C to 280°C at 12°C/sec for injector, 50°C to 330°C at 20°C/min for oven, and 250°C for ion source. Data were acquired at 17spectra/second, with electron ionization at -70eV. Over 300 annotations were generated, with about 140 metabolites identified in citrate- and EDTA-plasma using our in-house Binbase data processing system. Data was evaluated for instrument reproducibility by first considering data from one instrument, or individual instruments, over the course of the year, followed by comparing data across instruments at each given time point. Hardware-related factors include column or filament replacements, and mass spectrometer optimization. Separation or variations in data for known metabolites were identified to assess similarities and/or differences between instruments. In addition to hardware considerations, there are a few possible parameters in data processing that can influence data reproducibility, one parameter being selection of quant ion used in peak height calculation, especially in very high-abundant metabolites where the most abundant ion is selected for relative quantitation. Based on these findings, best strategies in handling such large samples to reduce batch effects are proposed. Analysis of technical replicates on near-identical instruments to evaluate batch effects typically observed over time on a single instrument.

POSTER 379

Minimizing unwanted variation in GC/MS and LC/MS metabolomics datasets

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Moderate and large scale metabolomics experiments necessitate grouping samples into batches, which are analyzed across several days or multiple instruments. Often it is impossible or impractical to distribute all sources of experimental variability into each analytical batch. A further challenge is that the analytical platform may not exhibit complete stability over time, resulting in signal drift within and across batches. Thus, data should preferably be normalized to mitigate batch effects in order to maximize the ability to reveal metabolomics responses to the parameters being tested. Towards this end, we obtained a detailed understanding of how different data preprocessing methods affect data quality and how to best minimize unwanted variation in GC/MS and LC/MS metabolomics datasets. We compared various batch normalization strategies for their ability to moderate among and within batch variability in GC/MS and

LC/MS metabolomics data sets. Normalization with internal standards, sample dry weight, common reference matrix material derived internally or externally to the experiment, and metadata mixed modeling were evaluated using both univariate and multivariate statistical analyses. We show that the outcome and interpretability of multivariate analyses of GC/MS and LC/MS data can be affected greatly depending on the data processing method(s) selected. Internally derived reference and metadata mixed model normalization approaches were effective in reducing the percent coefficient of variation for most metabolites among replicate samples. In addition, these two approaches resulted in lower within a across and within batch variation in multi-batch experiments as shown by principle component analyses and partial least squares regression analysis. Mixed modelling was particularly effective. However, it is important to guard against the presence of unique or highly variable metabolites in common reference samples, situations that can add variation to the rest of the data set. We conclude that careful experimental design that minimizes experimental biases coupled with rigorous data normalization that minimizes experimental biases not controllable by the experimental design results in optimal data matrices best suited for subsequent univariate and multivariate statistical analyses. Recommendations are given to minimize unwanted variation in metabolomics datasets. In depth comparison of data normalization schema reveals optimal protocol for large scale metabolomics data sets.

POSTER 380

The Advantages of Comprehensive Two-Dimensional GC-MS in the Analysis of Serum Samples for Metabolomics

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Compared to other mass spectrometric platforms, comprehensive two-dimensional gas chromatography mass spectrometry (GCxGC-MS, also known as 2D GC-MS) can provide superior separation capacity, selectivity, sensitivity, and dynamic range for the analysis of small molecule metabolites. However, GCxGC-MS is not widely used in metabolomics due to the difficulty of data analysis, making large scale studies impractical. New bioinformatics tools have recently emerged that greatly decrease the personnel time necessary to properly process GCxGC-MS data, making this approach feasible for large-scale metabolomics studies. This work examines the performance of GCxGC-MS vs. GC-MS in the context of a metabolomics analysis of a relatively large sample set. Human serum samples were obtained from subjects with a chronic neurodegenerative disorder (n=55) and age, gender, and ethnicity matched controls (n=55). Additionally, pooled serum QC samples were prepared. Samples were protein precipitated, dried, methoxyaminated, and derivatized with MSTFA. Processed samples were analyzed on a LECO Pegasus 4D GCxGC-TOFMS using GC-MS and GCxGC-MS analyses, under identical conditions, with a pooled serum QC sample being run every 10th biological sample. A 60 m DB-5MS column was used as the primary column and a 1 m DB-17MS column was used as the secondary column. Samples were run with an oven gradient starting by holding at 60 °C for 1 min, ramping at 5 °C/min, and holding at 290 °C for 12 min. Experimental data were processed with LECO ChromaTOF for spectrum deconvolution and metabolite identification by mass spectrum matching, while MetPP software was used for retention index matching, cross sample peak list alignment, data normalization, and metabolite quantification. The development and subsequent use of MetPP, used to align the ChromaTOF peak lists, made the previously impractical GCxGC-MS analysis of a large sample set practical. Qualitative examination of the spectra revealed areas containing coeluting compounds in the GC-MS spectra which were fully separated in the GCxGC-MS spectra. This real, physical compound separation reduces the potential for errors associated with in silico peak deconvolution by reducing the number of peaks needing deconvolution. Upon examination of an individual pooled QC sample, GCxGC-MS outperformed its GC-MS counterpart in terms of peaks detected as well as number of peaks annotated with a compound name through library searching. When processed at a typical signal-to-noise ratio (S/N) threshold of 50 (and a ChromaTOF similarity match threshold of 600), the GCxGC-MS run yielded 1517 peaks detected with 1011 of those peaks annotated with a compound identification, while the GC-MS run only yielded 505 peaks detected with 341 peaks annotated. When the whole data set (consisting of 109 experimental samples and 14 pooled QC samples) was processed, GCxGC-MS outperformed GC-MS,

with the final data sets consisting of 757 library-matched and aligned metabolites with GCxGC-MS and 254 with GC-MS. The increased number of aligned metabolites in the GCxGC-MS data set resulted in an increased number of statistically significant differentially expressed metabolites observed between the control and diseased subjects- with 34 found in the GCxGC-MS data set compared to only 23 metabolites observed in the GC-MS data set. We show the practicality of GCxGC-MS for metabolomics; offering improved separating capacity, selectivity, and sensitivity compared to GC-MS.

POSTER 381

An NMR Metabolomics Approach for Serum and Plasma Samples with Small or Variable Volumes

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Numerous metabolomics approaches focused on understanding, preventing, diagnosing and prognosticating of human diseases have been investigated using NMR and Mass spectrometry. NMR spectroscopy provides a powerful and relatively unbiased approach to metabolomics studies. However, high-throughput NMR detection of volume-limited biofluids remains challenging. The Southeast Center for Integrated Metabolomics (SECIM) offers global metabolomics and sometimes receives samples for which the optimal volume is unavailable, for example in blood from mice. We describe a strategy that we have developed to obtaining useful 1H NMR spectra from serum/plasma samples containing low and/or variable volumes. Three different groups were compared, intact human serum in 5mm NMR tubes, and protein precipitated human serum in 5mm and 1.7 mm NMR tubes with volumes ranging from 60 to 300 µL. Cold methanol was used to remove proteins from samples. After protein precipitation, supernatants were dried and resuspended in either 600 µL or 60 µL phosphate buffer in D2O and transferred to a 5mm or 1.7 mm NMR tubes, respectively. The strategy to remove proteins was applied for a study on mouse serum samples with variable volumes using 1.7mm NMR tubes. NMR experiments were performed on a Cryoprobe 600 MHz Bruker Avance II spectrometer. A 1D CPMG and/or NOESY presaturation pulse sequences were used for the experiments. Protein signals in intact samples were suppressed using the CPMG sequence, resulting in attenuating the concentration of metabolites bind to proteins. The PCA score plots elucidated that our strategy to remove proteins is effective in reducing the variation caused by proteins broadened the NMR peaks, which is more significant with lower volumes. In addition, PCA score plots illustrated using 1.7 mm NMR tubes were more effective to eliminate artificial variation caused by small and variable volumes. The strategy also allows a better metabolome coverage particularly for the samples with small volumes. Statistical analysis on the mouse study with variable volumes of 65 to 200 µL indicated that samples were separating base on their metabolome variation and not volume differences. This approach addresses practical issues obtaining high-throughput,1H NMR spectra to investigate and quantify serum/plasma metabolites with low volumes as 60µL.

POSTER 382

Volume determination with two standards: absolute quantification of metabolites by NMR from small volume samples. Application to Drosophila larvae

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The accurate measurement of metabolite concentrations in miniscule biological sample volumes is often desirable, yet it remains challenging. In many cases, the starting analyte volumes are imprecisely known, or not directly measurable, and hence absolute metabolite concentrations are difficult to calculate. Here we introduce Volume Determination using Two Standards (VDTS) as a general quantitative method for the analysis of polar metabolites in sub-microlitre samples using 1H NMR spectroscopy. This approach permits the back calculation of absolute metabolite concentrations from small samples of unknown volume. Where small sample volumes are also variable, VDTS can improve multivariate chemometric

analysis. As proof-of-principle, the VDTS-based method has been used to analyze polar metabolites in the hemolymph extracted from larvae of Drosophila fruit flies. Obtaining absolute metabolite concentrations requires normalization of the data on the basis of the extracted hemolymph volume. We obtained the extraction volume by indirect means using VDTS. Larvae were ruptured in a precise volume of saline containing a known concentration of 13C-labelled sodium formate. A precisely measured portion of the clarified solution was diluted into water containing 80 μM DSS and submitted to chloroform-methanol extraction. The polar fraction was evaporated to dryness and re-suspended in D2O. The NMR sample contains both DSS and 13C-formate as features that permit dataset normalization. The DSS concentration is essentially constant for each sample, Comparison of the DSS-normalised 13C-formate signal intensity between spectra obtained for real and mock-opened larvae allows for absolute metabolite quantification. 1H NMR spectroscopy can be used to provide a relatively unbiased approach to metabolomics investigation. Here we describe the development of methods for the application 1H NMR to the analysis of polar metabolites in small and variable samples of unknown starting volume. We select Drosophila larval hemolymph as a challenging test case and introduce the method of Volume Determination using Two Standards (VDTS) to assess accurately the sample dilution and original analyte volume. We report the concentrations of the most abundant polar hemolymph metabolites and provide a comparative analysis of the hemolymph metabolome under fed and nutrient restriction (NR) conditions in both male and female larvae. Application of VDTS combined with supervised fitting of the NMR spectra obtained for four different conditions (male vs. female, fed vs. NR) allows a quantitative assessment of each metabolite concentration, revealing previously unreported gender-specific variation of the hemolymph metabolome under fed conditions that is suppressed under NR. Moreover, it is demonstrated that standard application of PQN analysis to the raw data without correction for the sample-to-sample dilution factor fails to provide a physically self-consistent set of peak intensities in the loadings plot. However, by using VDTS to facilitate normalizing the data-set with respect to absolute concentration, the PCA output is rendered sensible. This approach allows for the facile determination of the sample-to-sample variance and, via analysis of the corresponding loadings plots, the identification of molecular species contributing to that variance. The principle of VDTS is thus demonstrated to be a valuable tool in metabolomics analysis of small and variable sample volumes, and is likely to be of general utility in measurement paradigms other than NMR spectroscopy. VDTS: a tool for small volume mixtures by 1H-NMR, and particularly beneficial when conservation of spectral mass is questionable.

POSTER 383

How to do “tandem MS” by means of in-source fragmentation for metabolite identification in untargeted metabolomics

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The role of non-targeted metabolomics with its discovery power is constantly growing in many different fields of science. However, its biggest advantage of uncovering the unexpected is turning into one of its biggest bottlenecks, particularly in metabolite identification. Proper identification of significant signals is of great importance and crucial to bring biological meaning to data; therefore particular attention has to be paid to ensure its high quality and certain level of confidence, e.g. through the MS2 or standards analysis. However MS2 is limited to certain types of MS analysers, therefore to overcome this, in-source fragmentation (ESI) has been used to fragment molecules and obtain product ions. In-source fragmentation by an enhancement of the fragmentor voltage results in the fragmentation of all compounds present in the sample, therefore the acquired spectrum contains many different signals. Consequently, determination of fragments corresponding to a compound of interest is challenging. Therefore a novel use of correlation analysis (CA) (testing Pearson product-moment (CA-P) and Spearman-rank (CA-S) methods) has been employed to select, among all detected signals, those that correlate with the molecule

of interest; assuming that its fragments will be strongly positively correlated ($r \geq 0.9$). This method together with the selection of the best threshold were tested on three different sample complexity levels: a single standard, mix of co-eluting standards and on a plasma sample. The use of this methodology requires previous putative identification as well as the fragmentation pattern for MS2. The ions found to correlate strongly with the precursor have to be compared with the fragments listed in the MS2 database. Using data acquired with the enhanced fragmentor, an EIC was obtained for each investigated metabolite and spectra from each point across the peak were exported. Spectra were merged, aligned and filtered. The final matrix, containing each m/z present across all scans, was used in CA (using CA-P or CA-S). CA successfully assigned fragments to their precursor ions, proving the suitability of 0.9 as a chosen threshold for correlation coefficients. The use of proposed methodology for quick differentiation between two metabolites with the same monoisotopic mass: Valine and betaine (m/z 118.0863) have been used as an example. Although each molecule has a different migration time (when analysed by CE), without MS2 or analysis of standards, they are not easily distinguishable. Since both molecules are different, fragmentation spectra obtained for them are different and easily distinguishable when compared to tandem MS spectra in databases. The use of developed methodology for semi-quantification of co-eluting metabolites with the same monoisotopic mass: Use of the proposed methodology is possible as long as there is one highly specific product ion for at least one of the co-eluting molecules which is absent for the second one. For example leucine (Leu) and isoleucine (Ile) give the same fragments except the product ion at m/z 69.07 which only appears in the spectrum for Ile. It was used to quantify the amount of Ile and Leu in a series of mixes containing different proportions of them. Calculated proportions are highly comparable to real ones. In-source fragmentation (by fragmentor enhancement) and novel of use of correlation analysis for metabolite identification by fragmentation pattern comparison.

POSTER 384

Metabolic profiling of the rat gut by 1H-NMR spectroscopy

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The large intestine is vitally important to human health. Also referred to as the “gut microbiome”, the large intestine contains more than 800 species of bacteria that help harvest energy, synthesize vitamins, and shape the immune system. Though it is critically important for human health and has been implicated in many diseases, the mechanism of metabolic communication between the gut microbiome and its human host is poorly understood. In this work, proton nuclear magnetic resonance is used to profile the metabolic signatures of rat stool water to provide insights into the functional segmentation of the rat colon microbiome. Changes in the metabolic profile are anticipated due to metabolism as well as transport of metabolites across the epithelium. The large intestine (colon) is surgically removed from the rat. Feces are removed from various positions of the colon, and a water extraction is performed. The stool water extract is flash frozen and stored at -80ºC prior to analysis. Each sample is analyzed using proton nuclear magnetic resonance spectroscopy (1H-NMR) on a Bruker 600 MHz NMR. 1H-NMR provides us with data to aid in the identification and quantification of metabolites present along the length of the colon. Ussing chamber experiments will be performed using cecum epithelial tissue removed from the rat colon. We will monitor the transport of metabolites across the epithelium by sampling both the lumen and serosal chambers and using 1H-NMR for detection. Previous work was conducted using ion-chromatography to identify and quantify inorganic metabolites. This work included over 120 stool water extract samples removed from various positions along the colon including the Ileum, cecum, proximal colon, mid-colon, and distal colon. Metabolites including, but not limited to, chloride, phosphate, sulfate, calcium, sodium, and potassium were identified and quantified to produce trends of metabolite absorption and secretion across the epithelium. This work brought upon the question of how organic metabolite composition changes along the length of the colon, which can be determined using 1H-NMR. 1H-NMR experiments have been conducted on approximately 80 stool water extract samples. Several metabolites have been identified including, but not limited to, acetate, butyrate, lactate, alanine, propionate, stachyose, leucine, isoleucine, valine, isovaline, and phenylalanine. The 1H-NMR spectra have been pre-processed and deconvoluted. Further data treatment is necessary including statistical methods such as principle least squares (PLS) analysis. Ussing chamber experiments have been conducted using epithelial tissue

removed from the rat cecum. The experiments utilize two chambers, one on each side of the tissue. In the chamber representing the lumen side of the tissue, a solution comprised of cecal stool is used, and on the serosal side, a parson's solution containing glucose is used. After 60 minutes, the solutions are removed and flash frozen until analysis. 1H-NMR analysis have been made and several metabolites have been observed on the serosal side including short chain fatty acids (SCFAs) acetate, butyrate, and propionate, giving us insight into the metabolites involved in transport. Further experimentation will include rate experiments, as well as experiments utilizing epithelial tissue removed from other various segments of the colon. Stool is surgically removed to provide insights into absorptive and secretory processes associated with the segmental stratification of the colon.

POSTER 385

Metabolite Profiling using Smart Isotope Tags and Ratio Analysis: NMR and MS Heterospectroscopy

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Urine is a convenient bio-fluid capable of providing a complete metabolic picture. Global metabolite profiling of human urine is, therefore, promising for the diagnosis of many human diseases. Given the large number of carboxylic acids in urine, it is of interest to develop advanced nuclear magnetic resonance (NMR) spectroscopy and mass spectrometry (MS) methods to better analyze the carboxylic acid sub-metabolome. Additionally, the difficulty in comparing and correlating metabolite data between NMR and MS limits efforts to exploit their combined strengths. Towards this goal, we are developing a new method, consisting of smart isotope tags and ratio analysis NMR and MS Heterospectroscopy (RANHSY), which we apply towards an enhancing metabolite profiling in human urine. Concentrated (5-10x) urine samples from healthy human volunteers were investigated to profile carboxylic acid metabolites. Metabolites containing carboxylic acid groups were separated from amines using an ethyl acetate extraction protocol (Peng and Li, Analytica Chimica cta, 2013, 803, 97). The first aliquots of extracted metabolites were tagged with a 15N-cholamine smart isotope tag (Tayyari et al., Anal. Chem., 2013, 85, 8715) and the second aliquots were tagged with 14N-cholamine in the same manner. 15N-tagged metabolites as well as standard compounds were investigated by 1H-15N heteronuclear single quantum correlation (HSQC) NMR spectroscopy at 800 MHz, and by LC-MS, while 14N-tagged metabolites were investigated by LC-MS only. We have previously shown that the smart tag approach provides an enormous increase in resolution and sensitivity for NMR based metabolomics analysis. However, amines can compete with 15N-/14N-cholamine in their reactions with carboxylic acids during the isotope tagging process, and their presence can create problems in both identification and quantitation of acid metabolites by NMR or MS. Extraction of acid metabolites into ethyl acetate at high salt concentration and low pH ensures the removal of amines. Extracted metabolites were tagged successfully with 15N-cholamine. Analysis of their 1H-15N HSQC NMR spectra showed as many as 2000 unique 1H-15N correlations, indicating ~1000 metabolites, assuming roughly two acid groups per metabolite. A majority of metabolites contain at least one carboxylic acid group that reacts with 15N-cholamine to generate 15N-amide bonds with unique 15NH peaks, with chemical shifts that depend on the metabolite structure. Importantly, the detection sensitivity gained herein is enormous as compared to the results obtained after the direct (without extraction) 15N-cholamine tagging. Interestingly, in many cases MS sensitivity of 15N-cholamine tagged metabolites was enhanced by nearly 3 orders of magnitude when compared to that obtained for non-tagged metabolites. This is due to the increased ionization efficiency imparted by the permanent charge of the metabolite tag. In a demonstration experiment, we applied the RANHSY method to NMR and MS methods, which enabled isolation of MS and NMR peaks from the same compounds. The high ionization efficiency of MS and enhanced sensitivity and resolution of NMR offered by the "smart isotope tag", 15N-cholamine, facilitates the collection of quantitative data, and connects the metabolite detected by MS to NMR data, and vice versa. Further analysis of both 15N- and 14N-tagged urine samples by LC-MS and the connection of NMR and MS data using RANHSY will also be reported. Isotope tagging and ratio analysis promise to improve

carboxylic acid detection for metabolite profiling and metabolite identification applications.

POSTER 386

Expanding the Limits of Human Blood Metabolite Quantitation using NMR Spectroscopy

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Metabolite profiling of human serum/plasma is of major interest for the investigations of virtually all human diseases. Nuclear magnetic resonance (NMR) spectroscopy is one of the two most widely used analytical techniques to analyze blood metabolites. Metabolite profiling of serum/plasma is generally met with two major challenges: first, the need to alleviate interference from the massive amount of serum/plasma proteins (6-8 g/dL); and, second, the need to unravel the inherent complexity of the mixture of compounds in the biofluid to reliably detect, identify and quantitate metabolites, individually. Further, the limited resolution and sensitivity combined with the challenges associated with unknown metabolite identification have restricted both the number and the quantitative accuracy of blood metabolite measurements. Pooled human serum was investigated using an array of 1D/2D NMR experiments at 800 MHz, after protein precipitation using various proportions of the organic solvents, methanol and acetonitrile, separately. Unknown metabolite identification involved a combination of literature/database searches, chemical shift, peak multiplicity and J-coupling measurements, and comprehensive 2D NMR spectral analyses. The putative new compounds were finally confirmed by spiking with authentic compounds. Chenomx NMR Suite Professional Software package (version 5.1; Chenomx Inc., Edmonton, Alberta, CA) was used to quantitate the metabolites. Peak fitting with reference to the internal TSP signal enabled the determination of absolute concentrations for identified metabolites in protein precipitated serum. Investigations of pooled human serum combining an array of 1D/2D NMR experiments at 800 MHz, database searches, and spiking with authentic compounds enabled the identification of 67 blood metabolites. Many of these (~1/3rd) are new compared to those reported previously as a part of the Human Serum Metabolome Database. In addition, considering both the high reproducibility and quantitative nature of NMR, as well as the sensitivity of NMR chemical shifts to altered sample conditions, experimental protocols and comprehensive peak annotations are provided here as a guide for identification and quantitation of the new pool of blood metabolites for routine applications. Hence, even non-expert NMR users can easily identify and quantify blood metabolites following the described approach, which is important for effective use of this tool. Further, investigations focused on absolute quantitation using organic solvents revealed a surprisingly poor performance for protein precipitation using acetonitrile. One third of the detected metabolites were attenuated by 10-67% compared to methanol precipitation at the same solvent to serum ratio of 2:1 (v/v). Nearly 2/3 of the metabolites were further attenuated by up to 65% upon increasing the acetonitrile to serum ratio to 4:1 (v/v). These results, combined with the newly established identity for many unknown metabolites in the NMR spectrum, offer new avenues for human serum/plasma based metabolomics. Further, the ability to quantitatively evaluate nearly 70 blood metabolites that represent numerous classes including amino acids, organic acids, carbohydrates and heterocyclic compounds using a simple and highly reproducible analytical method such as NMR may potentially guide the evaluation of samples for analysis using mass spectrometry. The ability to quantitate nearly 70 blood metabolites using 1D NMR signifies an important step for human serum/plasma metabolomics.

POSTER 387

Massive Glutamine Cyclization to Pyroglutamic Acid in Human Serum Discovered Using NMR Spectroscopy

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Glutamine is one of the most abundant metabolites in blood and is a precursor as well as end product central to numerous important metabolic pathways. A number of surprising and unexpected roles for

glutamine, such as the cancer cell glutamine addiction discovered recently, stress the importance of accurate analysis of glutamine concentrations for understanding its role in health and numerous diseases. To date, glutamine has been considered as a relatively stable metabolite under the mild conditions widely used in the analysis of human blood serum. In the present study we question this assumption, focusing on the stability of glutamine and its proclivity to form pyroglutamic acid. Intact, ultrafiltered and protein precipitated samples from the same pool of human serum were comprehensively investigated using 1H NMR spectroscopy at 800 MHz to detect and quantitatively evaluate blood glutamine levels. Metabolite assignments including identification of peaks from glutamine and pyroglutamic acid were made based on the recently published literature on unknown metabolite identification in human blood serum, which has enabled identification and quantitation of nearly 70 blood metabolites using 1D NMR [Anal. Chem. 87(1):706-15, 2015]. Chenomx NMR Suite Professional Software package (version 5.1; Chenomx Inc., Edmonton, Alberta, Canada) was used for absolute quantitation of the metabolites, glutamine and pyroglutamic acid. In the analysis of both ultra-filtered serum and protein precipitated serum using methanol, large signals that were assigned to pyroglutamic acid were observed. No such signals were observed in the intact serum NMR spectra. Quantitative analysis of the metabolites showed a massive glutamine cyclization to pyroglutamic acid; the cyclization causes a decrease in the apparent blood glutamine level by up to 75% and a proportionate increase in the levels of pyroglutamic acid. The magnitude of cyclization varied drastically for different samples from the same pool of human serum; however, the sum of the apparent glutamine and pyroglutamic acid concentrations in each sample remained the same suggesting that virtually all pyroglutamic acid was derived from glutamine cyclization. These unexpected findings indicate the importance of considering the sum of apparent glutamine and pyroglutamic acid levels, obtained from the contemporary analytical methods, as the actual blood glutamine level for biomarker discovery and biological interpretations. Interestingly, while glutamine cyclization occurs in both ultrafiltered and protein precipitated serum, the cyclization was not detected in intact serum. Recent mass spectrometry experiments have encountered the phenomenon of in source glutamate cyclization; however, NMR analysis does not cause this reaction, and hence enables accurate analysis of blood glutamine. Thus NMR spectroscopy provides a reliable method for the accurate analysis of glutamine level in metabolomics. Further, as observed for human serum in this study, glutamine cyclization may also occur during the analysis of other biological specimens as well, and hence caution is needed in the analysis of this biologically important metabolite. The contemporary methods of blood glutamine analysis need to account for the phenomenon of massive glutamine cyclization to pyroglutamic acid.

POSTER 389

Enhancing metabolomics: shining light on metabolism

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Current metabolomics methods are largely focused on NMR spectroscopy and mass spectrometry, usually with prior chromatographic separation. There are alternative methods based on the interaction of light with materials and whilst FT-IR spectroscopy has been used to generate metabolic fingerprints of samples, Raman spectroscopy has particular advantages as it is non-destructive, measurements with high molecular specificity can be made in aqueous environments, and this method can be coupled with microscopy to allow image analysis at very high spatial resolution (1 um is typical). We have been developing Raman spectroscopy for the analysis of biological systems. Raman spectra were collected using a DeltaNu Advantage 200A portable spectrometer (DeltaNu, Laramie, WY, USA), equipped with a 633 nm HeNe laser giving a power of 3 mW at the sample. In order to perform SERS silver nanoparticles were synthesized and characterized with UV-vis spectroscopy and electron microcopy and had typical diameter of 30 nm with narrow dispersion. For LC-SERS a Shimadzu LC-10AVP HPLC system (Kyoto, Japan) was coupled with a DeltaNu Raman spectrometer also with excitation at 633 nm. Raman spectroscopy has a rich history for the analysis of chemical species. However, whilst Raman spectroscopy offers unique specificity for molecular characterization the signal is usually rather weak. Fortunately this signal can be significantly enhanced using surface enhanced Raman scattering (SERS).

SERS involves coupling the analytes with a metal surface (roughed at the nano-scale and hence why colloids are used) during the Raman acquisition. Using judicious design of experiments we have recently demonstrated excellent detection and quantification for a range of drugs and biomarkers using SERS [Levene et al. (2012) Anal. Chem. 84, 7899-7905; Mabbott et al. (2013) Anal. Chem. 85, 923-931; Cowcher et al. (2013) Anal. Chem. 85, 3297-3302]. In this presentation we have further developed our SERS with chemometric and machine learning methods for multiplexed quantification of drugs and their metabolites [Alharbi et al. (2014) Analyst 139, 4820-4827] as well as developing LC-SERS for quantification of purines [Cowcher et al. (2014) Anal. Chem. 85, 9977-9984]. SERS provides quantitative analyses and LC-SERS allows absolute quantification of target analytes at levels lower or comparable to LC-MS.

POSTER 390

Development of a sheathless CE-MS interface for cationic metabolome analysis

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Capillary electrophoresis-mass spectrometry (CE-MS) is a powerful tool for comprehensive analysis of charged compounds. At present, sheath flow interface is the most commonly used technique in CE-MS. However, this interface leads to decrease in sensitivity due to dilution of analytes with sheath liquid. To improve the sensitivity with CE-MS, we have developed a novel sheathless CE-MS interface. A hole with 2 mm in diameter was drilled on a plastic plate (2 mm in thickness) and a cellulose acetate membrane (100 Da cut-off) was paste upon the hole. A fused-silica capillary was placed exactly on the hole and a small crack was made directly above the hole. The capillary was completely fixed with glue and external buffer reservoir was placed at the opposite side of the plastic plate and a platinum electrode was inserted in it. This sheathless CE-MS interface enables electrical contact between electrolyte in the capillary and external reservoir via membrane on the plastic plate. Under optimized conditions, 51 cationic metabolic standards were successfully separated and selectively detected using both a TOFMS and a triple quadrupole MS. With pressure injection at 50 mbar for 15 sec (15 nl), the detection limits for these metabolites were between 0.06 and 1.7 mmol/l for TOFMS, and between 0.003 and 0.4 mmol/l for a triple quadrupole MS. In addition, the developed method was applied to the analysis of cationic metabolites extracted from a small amount of mouse pancreas tissue (5 mg), and number of detected peaks was found comparable with conventional sheath-flow CE-MS methods. This interface could be a powerful new tool for highly sensitive CE-MS-based metabolome analysis.

POSTER 392

Profiling thiol metabolites and quantification of cellular glutathione using FT-ICR-MS

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Cellular thiol-containing metabolites are important for maintaining and regulating redox homeostasis. Studying the ratio of reduced/oxidized glutathione (GSH/GSSG) and quantifying the dysregulation of their levels are keys to understanding oxidative stress and the etiology of many human diseases. Current methods, though sensitive, cannot always be used for new metabolite identification and/or rely heavily on separation techniques that can limit their utility. We have developed a chemoselective method for the identification and quantification of thiol metabolites by direct infusion FT-ICR-MS. We illustrate our approach using the thiol-reactive, quaternary ammonium-based α−iodoacetamide probe, Quaternaryammonium Dodecyldimethyl Ethyl-iodoacetamide (QDE) and isotopologue *QDE. Rapid reaction of QDE with thiols helps identification of labile thiol-metabolites and

freezes thiol-disulfide flux, thereby, allowing accurate quantification of thiols and disulfides. The thiol-reactive, iodoacetamide-based probe QDE was synthesized for thiol-selective adduct formation. This probe features a hydrophobic domain to facilitate rapid extraction of adducts from the aqueous extracts to minimize matrix interference for accurate quantification and a quaternary ammonium moiety to greatly enhance ionization and subsequent detection of even low abundant thiol adducts by [+]-ion electrospray mass spectrometry. To further streamline the detection of thiol adducts, we developed a convenient protocol that employed both QDE and its labelled isotopologue in acetonitrile to lyse cells at pH 4 or 7.4 for effectively tagging thiol metabolites as isotopic pairs. The presence of isotopic pairs facilitated the identification of thiol metabolites from crude cell extracts and served as a standard for their accurate quantification. Addition of the ammonium α-iodoacetamide reagent QDE and its isotopologue *QDE directly to live cells followed by n-BuOH extraction and direct electrospray FT-ICR-MS analysis provides a registry of isotopologue pairs. The probe design and experimental protocol coupled with the high mass accuracy and ultra-high resolution of FT-ICR-MS enable determination of metabolite molecular formulae. Equally important is the enhanced chemoselectivity of QDE when cell extracts were reacted at pH 4, thereby better delineating thiol from non-thiol metabolites. Our analysis of A549 cell extracts revealed the major thiols to be glutathione, cysteine, and cysteinylglycine. We also observed, for the first time, S-alkylated hypotaurine. Finally, QDE and *QDE were readily applied for thiol quantification, such as demonstrated for the quantification of glutathione and glutathione disulfide. We showed this approach to be sensitive at the 40-nM range and linear in the concentration range of 0.04 to 5.0 μM with >99 % accuracy. Total concentrations of [GSH] and [GSSG] were determined as 34.4± 11.5 and 10.1±4.0 nmol/mg protein, respectively. In summary, the present approach should enable high sample and information throughput for profiling cellular thiols as well as facilitate their quantification. This method allows for selective and high-throughput identification of labile, low-abundant, thiol-metabolites and their accurate quantification using direct infusion FT-ICR-MS.

POSTER 393

Proton Elucidation for IsotoPe Analysis (PEPA): A proton-based fast screening approach for NMR-based stable isotope-resolved metabolomics

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Stable Isotope-Resolved Metabolomics (SIRM) makes use of isotope labeled substrates to trace back the incorporation of labeled atoms into the structures of a wide range of key metabolic intermediates. To date, NMR-based SIRM approaches rely on the use of 2D proton-based spectrally edited experiments such as 1H-1H TOCSY and/or 2D 1H-13C HSQC, requiring both very long acquisition times and skilled specialists to interpret the data produced. This has prevented the use of NMR-based SIRM in comprehensive metabolic analyses involving large number of samples. To overcome these limitations we have developed a new methodology called PEPA (Proton Elucidation for IsotoPe Analysis) which allows quick surveys to detect isotope stable enrichment from 1D-1H-NMR spectra readouts generated by NMR-routine metabolite profiling. U-2 OS osteosarcoma cell lines were used as a benchmark model to develop PEPA. Cells were subjected during 6 hours to serum deprived media prepared using uniformly labeled glucose ([U-13C]-Glc) or glutamine ([U- 13]C-Gln), or medium prepared with non-labeled substrates (in triplicate). Subsequently, cells were scraped and polar metabolites extracted. Crude cell extracts spectra were recorded at 298K on a Bruker Avance III 600 spectrometer operating at a proton frequency of 600.20 MHz using a 5 mm CPTCI triple resonance (1H, 13C, 31P) gradient cryoprobe. Metabolite resonances were resolved from 1D 1H-NMR spectra using a curve fitting-approach and PEPA was applied to predict fractional enrichment. 2D 1H-edited experiments (HSQC,HMBC,TOCSY and COSY) were acquired to confirm PEPA predictions. Using PEPA we determined significant fractional enrichment in 38 out of the 82 resonances identified in 1D-1H-NMR spectra from [U-13C]-Glc, [U-13C]-Gln experiments and their corresponding non-labeled controls. Fractional enrichment was calculated from the 1D- 1H-NMR central

peak area decay observed in cells cultured using 13C-labeled substrates when compared to their corresponding non-labeled controls. This central peak area decay is produced at expenses of incremental 13C-satellite peaks areas as consequence of 13C incorporation and displacement of non-labeled atoms. Noteworthy, all fractional enrichments predicted by PEPA were further confirmed using 2D-edited experiments. PEPA revealed isotope incorporation of 13C atoms in [U-13C]-Glc into the glucose anomeric carbon of UDP-Glucose, UDP-Glucuronate and UDP-GlcNAc, and into the riboside C1' carbon of these molecules. The same C1' carbon was also enriched in adenosine nucleotides showing in turn 13C- incorporation into their C2,8 adenine ring. Finally, labeled glucose was also significantly incorporated into lactate (CH); glycine(CH2); guanidinoacetate(CH2), aspartate(CH2), fumarate(CH), glutathione(CH2-glutamate) and glycerol(C1,3). Thus, using PEPA we demonstrated that in U-2 OS cell line, the carbon flow from [U-13C]-Glc was diverted through glycolysis, TCA cycle, PPP, glycine metabolism, hexosamine pathway and purine synthesis. On the other hand, using [U-13C]-Gln experiments PEPA found particularly high fractional enrichment levels in aspartate(CH2) and C5,6 of uracil in pyrimidine nucleotides demonstrating that diversion of aspartate to pyrimidine ring biosynthesis is markedly activated in this cell line. Additionally the fate of glutamine was found in glutamate(CH2), glutathione(CH2-glutamate) and succinate(CH2) indicating that glutaminolysis and biosynthesis of glutathione are also activated. Altogether PEPA greatly simplifies flux monitoring using stable isotope tracers becoming of major interest in cancer cell metabolism studies. Of mention, PEPA outputs complementary information to this obtained using 2D NMR-based SIRM approaches allowing a quick screening of the labeled substrates fate in larger sample sets. PEPA allows quick and easy monitoring of flux through different pathways using multiple 1D-1H-NMR readouts generated by NMR-routine metabolite profiling.

POSTER 394

Untargeted Stable Isotope Tracing: Establishing A Novel MS-based Strategy for Discovering Metabolic Fate and Flux

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Metabolomics has proven to be a powerful tool for probing complex biological mixtures to discover changes in metabolite levels that arise from drug treatments, gene mutations and disease states. However, such metabolite profiling experiments typically provide only a snapshot of cellular metabolism; devoid of information on the dynamics and flux of metabolites within metabolic networks. Stable isotope tracing has been useful for elucidating metabolic fluxes through biochemical pathways and networks, however this approach has been either limited to study of predicted tracer-derived metabolites or requires complex mathematical models and assumptions for estimation of isotope incorporations. Here we describe a simple workflow, requiring no preconceived knowledge of pathways, to enable untargeted fate tracing of a stable isotope labeled molecule of interest. The workflow uses commercial metabolite profiling software (Agilent MassProfinder 6.0, Mass ProfilerProfessional 13.0 and MassHunter 6.0) for untargeted identification of differentially-expressed metabolites in cultured cells, grown in heavy isotope media, compared to light isotope media. For metabolites significantly increased in heavy isotope group, curated isotopologues (M-1, M-2, …M-6) were generated by subtracting the accurate mass of the metabolite by one to six (or higher number) heavy isotope elements (e.g. [13C] or [15N]). Similarily, curated isotopolgues of M+1, M+2, M+3…M+6 were generated by adding one to six heavy isotope elements to metabolites significantly decreased in heavy isotope group. Extracting ion abundance of curated isotopologues at the same retention time of their respective parent ions provides isotope distribution of incorporated metabolites Untargeted isotope tracing workflow was tested in HT-29 colon cancer cells cultured with 2 mM acetate (2[13C]) for 3.5 h. Acetate was found to incorporate into acetyl-CoA, TCA cycle and its cataplerotic reaction intermediates, amino acids, peptides, glutathione, glutathione disulfide, acetylated metabolites, acylcarnitines, short and long-chain fatty acids and phospholipids. Quantification of relative isotope incorporation reveals that acetate contributes to ~ 63% of the total citrate pool, 70% of short chain fatty acid and acylcarnitine pool, outcompeting glucose for metabolite synthesis. Despite an ample supply of amino acids in the cell culture media, acetate contributed to 20-36% of glutamate, aspartate, proline and their downstream peptides. With the exception of a some acetylated metabolites that show only 2[13C] incorporations (e.g. acetyl-CoA, acetyl-carnitine, acetyl-serine, UDP-acetyl-

glucosamine and acetyl-neuraminic acid), numerous metabolites revealed multiple and greater number of [13C] incorporation, including butyrate and butyrylcarnitine which show ~40% of 4[13C] incorporations. Incorporation of multiple [13C] correlates with a faster acetate metabolic flux – results suggest that acetate and acetate-derived butyrate might be important and primary bioenergetic source for colonocytes. Overall, our results demonstrate a highly-effective and broadly-applicable approach for metabolic fate tracing. We confirm that colon cancer cells are active consumers of acetate and, applying this new untargeted stable isotope tracer approach, quantitatively define the disparate metabolic fates of acetate-derived acetyl-CoA. Untargeted stable isotope tracing provides a powerful tool to discover the fate of a broad range of the tracer-derived metabolites.

POSTER 395

Accurate mass GC-QTOFMS with chemical ionization for isotopologue and tandem mass isotopomer analysis of primary metabolites in biological samples

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In the past two decades 13C based metabolic flux analysis (13C-MFA) of microbial organisms has become a key analytical technology especially in support of metabolic engineering [Sauer U., Mol.Syst.Biol, 2(2006)]. The introduction of stable isotope tracers, like 13C, into an organism, leads to a characteristic pattern of labeled metabolites, which are most frequently measured by NMR or MS based techniques [Zamboni N. et al.,Nat.Protoc.,4(2009)]. In this context, the principle of orthogonality is essential and can be additionally achieved when employing GC and LC. In the present work, we have developed a novel analytical approach based on accurate mass GC-QTOFMS for analysis of isotopologue and isotopomere fractions of amino acids, organic acids, sugars and sugar phosphates in biotechnological samples. An Agilent 7200 GC-QTOFMS system was used for the analysis of biotechnological samples from a 13C-based metabolic flux experiment. A two-step derivatization was performed via ethoxymation and trimethylsilylation. Both chemical ionization and electron ionization were employed; collision induced dissociation was achieved by using N2 as collision gas. Data evaluation included isotope interference correction for the contribution of natural abundant isotopes from the derivatized groups and the native molecule itself employing an object-oriented software tool based on combinatorics, called “isotope correction toolbox“[Jungreuthmayer C. et al. in preparation]. As a matter of fact the validation of the novel GC-QTOFMS based methodology is essential for further application in metabolic engineering and industrial biotechnology. The GC-QTOFMS method has been optimized in terms of derivatization, ionization modes as well as fragmentation settings. The data obtained by the accurate mass GC-QTOFMS method under different experimental conditions are compared in terms of precision and trueness, as well as with an orthogonal technique. Chemical ionization gave the protonated molecule enabling selective fragmentation of isotopologues and the unambiguous assignment of isotopomeres to the respective isotopologue. Due to the information gain when employing chemical ionization with consecutive collision induced dissociation, GC-CI-QTOFMS proved to be most valuable for the analysis of tandem mass isotopomer distribution of primary metabolites. The application of electron ionization showed in general higher sensitivity and lower limits of quantification. As a drawback the most sensitive fragments did not contain the complete carbon backbone limiting the applicability of electron ionization in the context of 13C-MFA. Based on biotechnological samples, the influence on 13C-MFA results, when applying a threshold and/or weighting to the interference corrected isotopologue and isotopomer distribution is discussed. It is demonstrated that the application of high resolution mass spectrometry is highly beneficial in terms of unambiguous identification of fragments, influencing the outcome of isotopomere distributions, especially in biological samples. A novel GC-CI-QTOFMS based approach for accurate determination of isotopologue and tandem mass isotopomer fractions is presented.

POSTER 397

Improving local false discovery rate in LC/MS mebabolomics data by incorporating feature reliability

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Feature selection is a critical step in translational research using high-throughput methods. False discovery rate (FDR) is an important tool of statistical inference in feature selection. It is commonly used to screen features (SNPs, genes, proteins, or metabolites) for their relevance to the clinical outcome under study. Traditionally, all features are treated equally in the calculation of global FDR or local fdr (lfdr). In many applications, different features are measured with different levels of reliability. In such situations, treating all features equally will cause substantial loss of statistical power to detect differentially expressed features. Feature reliability can often be quantified based on the measurements. Here we present a new method to estimate the lfdr that incorporates feature reliability. We also propose an index to measure reliability for metabolomics data with repeated measures. The reliability index aims to account for within-subject variation, by calculating the standard deviation of non-zero observations of each subject. The lfdr is then estimated via 2-dimensional kernel density smoothing methods (fdr2d), using t-statistics obtained from simultaneous linear models of a metabolome-wide association study (MWAS) and the reliability scores. All methodology and analysis was run in R 3.1.0 and pathway analysis was carried out in MetaboAnalyst 2.0. We compared our fdr2d method to traditional 1-dimension lfdr methods. In simulations, our method achieved better balance between sensitivity and controlling false discovery, as compared to traditional lfdr estimation. More truly differentially expressed features were detected, while false discoveries were not overly conservative. Next, we analyzed a real metabolomics dataset, consisting of patients who have undergone coronary angiography to document the presence/absence of coronary artery disease (CAD). Our outcome of interest was High Density Lipoprotein (HDL). The HDL levels ranged from 5 – 95 mg/dL, with mean 42.3 mg/dL and standard deviation 12.8 mg/dL. We ran an MWAS on HDL, adjusting for batch effect and applied our fdr2d method. Results were compared against a 1-dimensional lfdr (fdr1d) method that uses only the t-statistics from the MWAS. The fdr1d method also employs kernel density smoothing. In this dataset, the reliability indices ranged from 0.0417 – 1.044. The fdr2d method detected more differentially expressed metabolites that are biologically meaningful as compared to the fdr1d method (378 and 139 respectively). Most of the features detected by the fdr1d method were also detected by the fdr2d method. Pathway analysis revealed more pathways of potential interest under the fdr2d method, including D-Glutamine and D-glutamate metabolism (p=0.006), which was the only one indicated by the fdr1d method (p=0.015). The proposal of a new reliability index and its use in 2-dimensional lfdr estimation detects more differentially expressed metabolites.

POSTER 398

Ultra-Performance Liquid Chromatography-Tandem Mass Spectrometry Profiling of Steroid Metabolome in Human Saliva

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In humans, steroids play a broad and vital role in regulation of gene expression, secondary sexual characteristics, maturation, reproduction, cardiovascular health, neurological functions, etc. Measurement of steroids in biological samples is essential to monitor human health. Because of the blood streams connection to saliva production, salivary steroid concentrations can be correlated to serum steroid concentration, making saliva a noteworthy alternative to evaluate systemic steroidogenesis. Clinically,

saliva collection has the potential to provide a high-throughput, non-invasive, and inexpensive technique to evaluate steroid levels. However, global steroid metabolism in human saliva has not been investigated previously. Therefore, we have created a steroid metabolome profile of human saliva. We recruited a total of 50 male and females ranging from 18 to 35 years of age to provide a saliva sample and complete a questionnaire. The questionnaire collected information on age, ethnicity, sex, supplements, medications, and diet. Subjects were instructed not to eat, chew gum, brush their teeth, or drink anything besides water prior to sample collection. Saliva samples were extracted, concentrated, and filtered before being introduced into a Waters Acquity UPLC system connected to a Xevo-TQ triple quadrupole mass spectrometer (UPLC-MS/MS). The resulting UPLC-MS/MS data was analyzed and processed using MassLynx 4.1 software. UPLC-MS/MS results show the presence of many metabolites from various steroid classes in human saliva. Particularly we were able to measure progestins, androgens, estrogens, glucocorticoids, mineralcorticoids, neurosteroids, bile acids, and oxysterols. Human health can be monitored using the salivary steroid metabolome profile identified through this study.

POSTER 399

Synthesis of Carbohydrates as Reference Standards for Metabolomics Studies

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The Metabolite Standards Synthesis Center (MSSC) at SRI International provides synthetic chemistry services to the NIH Common Fund's Metabolomics Program. Metabolomics researchers are encouraged to nominate compounds online at the Metabolomics Workbench (metabolomicsworkbench.org/standards). As part of this program, SRI has made available labeled and unlabeled carbohydrates to the community, including 2-keto-3-deoxy-D-gluconic acid, 3-deoxyarabinohexonic acid, 13C6-glucose 6-phosphate, 13C6-fructose 6-phosphate, 13C6-phosphogluconolactone, 13C6-phosphogluconate, 13C5-ribose 5-phosphate. Synthesis of other carbohydrates is in progress. The Metabolomics Workbench houses data for synthesized compounds and receives requests for aliquots to be provided to researchers. Compounds are prepared by multi-step syntheses using chemical and enzymatic reactions as appropriate. Synthetic procedures and routes are adapted from the literature to incorporate modern methods, enable isotopic labeling, maximize chemical yields, and simplify purification. Acidic compounds are typically stored as calcium or barium salts to maximize stability. Compounds are analyzed by HPLC with ELS detection and characterized by NMR, mass spectrometry, and other techniques as necessary. Aliquots of 2-keto-3-deoxy-D-gluconic acid, 3-deoxyarabinohexonic acid, $^{13}C_6$-glucose 6-phosphate, $^{13}C_6$-fructose 6-phosphate, $^{13}C_6$-phosphogluconolactone, $^{13}C_6$-phosphogluconate, $^{13}C_5$-ribose 5-phosphate are immediately available to the community. Additionally, $^{3}C_7$-sedohepulose 7-phosphate, $^{3}C_4$-erythrose 4-phosphate, $^{3}C_5$-xylulose 5-phosphate, and $^{3}C_5$-ribulose 5-phosphate are being prepared in our labs for this program. 13C-lableled phosphogluconolactone, phosphogluconate, ribose 5-phosphate, sedohepulose 7-phosphate, erythrose 4-phosphate, xylulose 5-phosphate, and ribulose 5-phosphate are new compounds.

POSTER 400

NMR and liquid chromatography mass spectrometry method development for clinical metabolomic profiling of saliva samples

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Most metabolomics studies employ techniques such as nuclear magnetic resonance (NMR) spectroscopy or mass spectrometry (MS) for profiling and/or quantifying metabolites. NMR is a robust, reproducible and high throughput analytical technique for metabolic profiling. 1H NMR and 2D experiments allow for correct metabolite assignment and quantification. Liquid-chromatography (LC) coupled to MS-based platforms have the advantage of detecting metabolites at lower levels (pM) than NMR. Bioactive lipids such as oxylipins and endocannabinoids are an important subset of the metabolome. These compounds are uPoster or down-regulated in different heath conditions, environmental exposures or type of diet. Saliva is a metabolic-sensitive biofluid, which can be easily and non-invasively collected making it a good candidate for lipidomic targeted LC-ESI-MSMS method and NMR spectroscopy metabolomics. Saliva was collected from spit, chewing pad and parotid gland suction (volunteer sampling). An untargeted and targeted analysis of the NMR experiments was performed. For the later, the samples were filtered to remove high molecular weight molecules (e.g. proteins) and diluted in phosphate buffer (with sodium azide, 10 % D2O and TMPS as the reference), followed by data acquisition and metabolite quantification by using the Chenomx NMR suite®. Multivariate analysis of binning data for the unfiltered samples was used for the untargeted approach. For LC-ESI-MSMS analysis raw saliva and filtrated saliva samples, were processed by one single solid-phase extraction (SPE) step and endocannabinoids and oxylipins were quantified by validated ultra-performance liquid chromatography coupled to electrospray tandem mass spectrometry (UPLC-ESI-MS/MS) methods. NMR profiling lead to the quantification of 33 metabolites with levels from 0.0013 to 5.51 mM. Overall, the highest metabolite level was urea was found in the parotid gland samples. This concentration decreases markedly in the saliva collected from chewing pad and spit ((5.51 to 1.82 mM). This observation may be justified by hydrolysis to carbon dioxide and ammonia by bacterial ureases of acids in the oral environment. Acetate, which curiously shows the second highest levels, follows a different trend. The concentration for this metabolite is higher in the chewing pad and spit when comparing to the parotid sample (from 3.2614 to 0.0383 mM). Once more, this can also be explained by oral bacterial activity. >UPLC-ESI-MS/MS methods included endocannabinoids (N=15) and oxylipins (N=42). For each standard the MS/MS parameters, such as mass transitions and collision energies, were optimized in order to obtain lower quantification limit (LOQ) possible and highest MRM sensitivity and selectivity. LOQ for endocannabinoids metabolome were in the range 0.5-1000 fg and for oxylipins Linearity and validation parameters were determined according to FDA guidelines. Separation of critical pairs of isomers presenting the same MRM transitions and known to be difficult to separate with a large number of analytes in the same run was achieved for the following isomers 2-AG/1-AG, PGE2/PGD2 and Resolvin D1/D2. Recovery rates for deuterated internal standards are comparable to plasma matrixe. Endocannabinoids, especially N-acetyl ethanolamides (AEA, OEA and PEA) have been reported with significantly differences in obese vs healthy humans. Linoleic acid epoxides from the CYP pathway were detected in saliva samples. This NMR method is currently being employed in the study of 25 year longitudinal project base on the Betula biobank were aims to profile and identify patients diagnosed with Alzheimer’s Disease (demented and pre-diagnosis). Method development for NMR and LC-ESI-MSMS quantitative profiling on human saliva.

POSTER 401

Development of Rapid Microbore Metabolic Profiling (RAMMP) UPLC-MS Approaches for High-Throughput Phenotyping Studies

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As metabolic phenotyping moves from small scale “proof-of-principle” studies to much larger scale biomarker discovery and patient stratification applications, rapid and economical analysis demands robust, high-throughput methods. While direct infusion mass spectrometry (DIMS) permits rapid analysis, it suffers from the inability to distinguish isobaric/isomeric metabolites and an increased risk of ion

suppression. UPLC-MS is a well-established platform for metabolite profiling, but microscale chromatography is one approach to reduce the amount of sample and solvent consumed, thereby minimizing expense and environmental impact, but is yet to be widely adopted. A rapid microbore metabolic profiling (RAMMP) UPLC-MS method has been developed to provide a high-throughput analytical platform for metabolic phenotyping from large sample cohorts without the need for multiple batches of analysis. Urine samples were diluted (1:3) with acetonitrile for protein removal, vortex mixed and centrifuged at 10,000 g (10 min). The supernatant was aliquoted into 96-well plates and further diluted (1:9) with water. Samples were analysed using a reversed-phase UPLC system coupled to a Synapt G2-S HDMS mass spectrometer in positive and negative electrospray (ESI) modes. Samples were analysed by a conventional method (2.1 x 100 mm column) operated at 0.5 mL/min and the RAMMP method (1 x 50 mm column) at 0.4 mL/min. Pooled quality control (QC) samples were injected at the start of each analysis and throughout the run to assess analytical reproducibility. Data was processed using Progenesis QI software and principal components analysis (PCA) performed using SIMCA-P software. A conventional reversed-phase UPLC-MS method for urinary profiling was scaled to provide a rapid gradient approach based on microbore chromatography with a run time of 2.5 min/sample. Despite a reduction in peak capacity (50 compared with 150) and ions detected (ca. 19, 000 compared with ca. 6000), the RAMMP method achieved similar levels of group discrimination to conventional UPLC-MS when applied to rat urine from two toxicity studies (2-bromophenol and acetaminophen), with the same features of significance responsible for discrimination of the different sample groups conserved between both analytical platforms. Robustness and repeatability of the assay over 500 injections (ca. 20 hrs) showed a modest reduction in repeatability for the RAMMP method in comparison to the increased sample throughput, with 82 % of the total features having a CV of <30% compared with 89 % generated by the conventional method. The method was used to analyze 800 samples derived from chronic acetaminophen toxicity to not only increase throughput but also eliminate the need for multiple sample batches. Urine samples were prepared in 384-well plate format and analysed in a single batch. Results showed tight clustering of QCs and clear differentiation of the dose groups and, although a sacrifice to resolution is expected, acetaminophen and its major metabolites, which share a common fragment ion (m/z 152.0712), were still resolved by the RAMMP method. The combination of reduced column diameter and length with increased linear velocity and high pressures provided a five-fold reduction in analysis time, and a ten-fold reduction in sample consumption. It can be concluded that this RAMMP approach, providing robust and sensitive analysis, is well suited to high-throughput metabonomic studies of complex mixtures such as urine while maintaining analytical integrity, reducing sample and solvent requirements and overcoming analytical drift associated with lengthy analysis times. Development of a robust, rapid metabolic profiling screening approach; utility of microscale chromatography for large scale metabonomic studies

POSTER 404

Profiling Endocannabinoids and Cannabinoid Receptor Agonist/Antagonist Fatty Acid Amides Using UPLC-TOF Ion Mobility Mass Spectrometry

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The endocannabinoid system is responsible of neuro-modulation of variety of neurophysiological processes, including motor learning, synaptic plasticity, appetite, pain sensation, and addiction. Endocannabinoid receptors, CB1 and CB2, and phospholipid-derived endocannabinoids, anandamide (AEA) and 2-arachidonoyl-sn-glycerol (2-AG), are widely studied. Recently, number of fatty acid amides (FAAs) have been identified to modulate endocannabinoid system as agonist of CB1/CB2 or inhibitors that both block the endocannabinoid system as a cannabinoid receptor CB1 antagonist, and elevate AEA levels by inhibiting its degradation or uptake in the brain cells. Profiling of endocannabinoids and structurally similar endogenous FAAs using UPLC-TOF ion-mobility mass spectrometry provides an opportunity to comprehensively study the mechanisms associated with the modulations in the endocannabinoid system. Chromatographic and gas-phase ion-mobility separation of endocannabinoids and FAAs was achieved on an Acquity UPLC coupled SYNAPT G2Si mass spectrometer using a reversed-phase gradient separation on a BEH C18 (2.1x150 mm) column with MS data acquisition in HDMS mode. Quantitative assessment of 18 endocannabinoids and FAAs in mouse brain and human

plasma was performed using a standard addition method. A 50 mg of mouse brain homogenate prepared in 50:50 acetonitrile:water and 200 µl of human plasma were separately spiked with isotopically labeled internal standards and extracted using a solid phase extraction (SPE) on a Waters Oasis HLB cartridge. Chromatographic peak-area ratios of endocannabinoids/FAAs and their stable-isotopically-labeled versions (when available) were used to construct a concentration response curve for quantification. Endocannabinoids and FAAs represent a class of structurally similar metabolites, some of which are isobaric positional isomers and difficult to resolve chromatographically using state-of-art UPLC methods. Using ion mobility (IM)-based gas-phase separation, an orthogonal technique to chromatographic separation, we have taken advantage of the differential mobility drift times to separate positional isomers such as [5(6), 8(9) and 11(12)] of EET ethanolamides, and 2-AG from 1-AG and quantify them in a high-throughput manner. An optimized sample preparation method and the UPLC-TOF-IM MS method is used to quantify18 endocannabinoids and FAAs in mouse brain tissue and human plasma, and profile additional 12 FAAs deemed important in the modulation of endocannabinoid system. QC samples for a select number of endocannabinoids and FAAs spanning the low, mid, and high range of the calibration curve are used to assess the variation in accuracy and precision of QC and calibration standards. NIH Grant 1U24DK097193. Ion mobility enhanced separation and measurement of endocannabinoids and FAAs provides an opportunity for in-depth probing of the endocannabinoid system.

POSTER 405

Sample Preparation for Global Metabolomics of Human Plasma by LC-MS: Lessons from Recovery, Matrix Effects and Correlation Analysis Studies

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Solid-phase extraction, liquid-liquid extraction methods and sequential extraction methods are currently widely underutilized in global metabolomics analysis of plasma even though they can enhance the detection of low abundance metabolome. To develop the most orthogonal combinations of methods and improve sample preparation for untargeted metabolomics of plasma, it is important to evaluate not only metabolite coverage and extraction reproducibility, but also recovery and ion suppression. The objective of this work was to develop two new sample preparation strategies (size-exclusion and SPE) and systematically evaluate the performance of these newly developed methods against solvent precipitation, liquid-liquid extraction with MTBE, C18 SPE and ion-exchange SPE with respect to metabolite coverage, method precision, absolute recovery in plasma and standard solutions and ion suppression. Systematic evaluation of sample preparation methods was performed using standard mixture of metabolites spanning logP between -3.9 and 8.6. Spiked standard analytes were extracted from buffer or plasma using solid-phase (divinylbenzene-pyrrolidone (PEP), octadecyl (C18), divinylbenzene-cation-anion exchange (IEX)); size-exclusion (SE); solvent precipitation (methanol, methanol/ethanol and methanol/ MTBE (methyl-tert-butyl ether blends 1/1 (v/v)); and liquid-liquid extraction (MTBE) methods. Samples were analyzed using mixed-mode ion-exchange-octadecyl chromatography and octadecyl reversed-phase chromatography using positive and negative electrospray ionisation (ESI) and high-resolution mass spectrometry. Metabolite recovery in plasma was determined using standard addition calibration method for each extraction method tested. Ion suppression effects were evaluated by comparing signal of post-extraction spikes to that of standard solutions across minimum of three concentration levels for all methods. The results allowed us to assess systematically the selectivity and recovery of different extraction methods. Liquid phase extractions of standard metabolites from buffer and plasma demonstrated quantitative and reproducible recovery of the majority of tested metabolites. We confirmed that methanol and its blends provided good extraction capability toward all tested metabolites independent of their polarity. Notably, the recovery of peptides was significantly decreased in solvent precipitations. We show that size exclusion approach can address this limitation by quickly separating peptide and metabolite fractions in plasma and buffer while maintaining good metabolite recovery across various metabolite classes. MTBE showed enhanced selectivity for species with LogP > 0.5 which indicates it can be used in the removal of hydrophobic compounds without significant compromise towards recovery of polar species in sequential sample preparations. Solid phase extractions showed compound specific complementary selectivity in

both plasma and in buffer. Thus, C18, ion exchange (IEX) and polar (PEP) extractions quantitatively recovered several metabolites and demonstrated polarity and/or class-dependent selectivity. The usage of standard addition calibration method allowed us to detect analytes whose signals were influenced by extensive matrix effect or co-eluting isobaric interferences and to characterize more precisely the absolute recovery and reproducibility of standard analytes between methods in a systematic manner. Finally, a novel approach using correlation analysis for the characterization of selectivity of extraction methods towards unknown metabolites using absolute recovery of standard analytes in the same samples was proposed and evaluated. We were able to demonstrate for the first time the correlation in inter-method recovery of various analytes in plasma and a possibility to separate them into groups with distinct recovery patterns. This result enabled us to expand systematic evaluation of method selectivity towards unknown signal features and provides the basis for more rational method selection for global metabolomic analysis. This is first systematic evaluation of recovery, matrix effects and expanded characterization of method selectivity for global metabolomics of plasma.

POSTER 406

Fishing for metabolites: chemical derivatization approaches for extracting metabolites from extreme environments

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Our world is dominated by microbes that carry out critical processes for life and that are responsible for shaping the biosphere, including the Great Oxygenation Event, which was driven by early cyanobacteria. Many microbial communities reside in extreme environments of high temperature, mineral content or salinity, making the assessment of metabolic exchange among community members difficult. We are developing approaches for extracting metabolites from these environments using chemical derivatization and report here a novel approach based on dansylation of amino acids in sample matrices containing up to 2 M magnesium sulfate. This method has shown advantages in terms of sensitivity and specificity when determining the amino acid content of extreme environments. For method optimization, 40 µg of amino acids in water were combined with 1 mg of dansyl chloride in acetonitrile and diluted to a final volume of 1.2 mL with 0.5 M sodium carbonate-sodium bicarbonate buffer (pH 9.5). The reaction solution was mixed using a benchtop shaker at 60°C for 15 min, and a liquid-liquid extraction was then performed using dichloromethane and water. The organic layer was collected and reconstituted in 2 mL of water and acetonitrile (95:5, v/v) followed by online SPE-nanocapillary LC-MS/MS analysis at a flow rate of 300 nL/min. Online SPE was performed using a tri-valve system with a microSPE column coupled in line with the reversed-phase capillary LC column for sample enrichment and matrix elimination. We analyzed unlabeled and stable isotope-labeled (13C, 15N) amino acids using the dansylation approach in order to optimize the measurement of these metabolites in extreme environments. Both the native and 13C, 15N-labeled amino acids were successfully derivatized and extracted from buffer containing 2 M magnesium sulfate using the present workflow. MS/MS fragmentation studies revealed that most of the amino acid derivatives shared similar fragmentation pathways, while the resulting fragment ions themselves can be unique depending on which amino acid derivative was analyzed. The unique fragment ion from each singly charged, dansylated amino acid derivative was used for quantification purposes, and a fragment ion common to all dansylated amino acids provided identification confidence. The effect of pH during extraction was studied and was shown to have a critical effect on the analyte partition between aqueous and organic layers, which in turn impacted the extraction yields and observed MS signals. The dansylation reaction time was optimized to be 15 min for most of the amino acids studied. We propose that this method can be readily extended for other amine- or phenol-containing analytes in extreme environments, such as high salt. The optimized method will be demonstrated in the characterization of metabolic exchange in co-cultures of cyanobacteria and heterotrophic bacteria. A novel workflow was developed to measure polar metabolites in high salt matrices.

POSTER 407

Solid phase extraction for complete metabolite recovery from salty samples

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Salts present in samples for mass spectrometry present a large problem, as they ionize extremely well, carry over across multiple samples, contaminate the MS surfaces and suppress the signal of other compounds. Media from bacterial isolate samples is often high in salts, making exometabolomic analysis of these samples difficult. Many methods for salt removal involve organic extractions, which along with crashing out salts, lead to the precipitation of polar compounds, resulting in their loss. In this study, solid phase extraction (SPE) cartridges composed of a styrene-divinylbenzene polymer with a non-polar surface were used to effectively remove all salt from media samples from the Great Boiling Spring in Nevada, while retaining all polar metabolites. SPE cartridges (1 mL volume) were pre-equilibrated with 3 mL of MeOH followed by 5 mL of H2O. The cartridge was dried with air, and a mixture of polar metabolites in NaCl, R2A, BMR or media containing a hot spring extract were then acidified with HCl and flowed through the cartridges. The cartridges were then rinsed with 2 mL of 0.01M HCl. To elute the de-salted metabolites, cartridges were rinsed with 2 mL of MeOH followed by 2 mL of ACN, and the pooled eluent collected and dried down. Dried extracts were re-suspended in MeOH containing internal standards. Samples were filtered and run on pHILIC column with an Agilent 1290 UHPLC coupled to a QExactive mass spectrometer. All samples were run without salt removal and compared with samples passed through the SPE cartridges, with and without a wash step. For the R2A, BMR and hot spring extract media samples, the SPE with washing allowed for retention of many metabolites while removing all salt from the samples. Samples run without salt removal contained large peaks for two different salt clusters. In these regions of the run, no metabolites were visible. Those same regions in samples which had been filtered/washed with the SPE cartridges contained hundreds of features, including numerous polar amino acids, and unknown features. Direct comparison of the intensities for some annotated metabolites demonstrated increases of 1.2 – 150 fold in adenine, adenosine, threonine, valine, leucine, phenylalanine, and tyrosine upon salt removal with the PPL cartridges. Unfortunately, some of the more hydrophilic amino acids showed decreases in intensity (2-10 fold) upon SPE cartridge filtration, however were still significantly above the detection limit. Overall, filtration of acidified salty media samples through SPE cartridges composed of a styrene-divinylbenzene polymer with an acidified wash step allowed for successful removal of all salts from media samples with partial or complete retention of most metabolites. Percentage recovery is currently being calculated for the salty standard mixtures, and the washing protocol is undergoing optimization in attempt to retain more of the hydrophilic compounds. This is the first report of a method to effectively remove salt from metabolomic samples while retaining polar/hydrophilic compounds.

POSTER 408

Development of in-SPE derivatization method for GC-MS metabolome analysis

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Due to complex and long-time sample preparations, including solvent extraction, centrifugal concentration, freeze-drying and derivatization, technical skills have been required in a conventional

metabolome analysis for GC-MS. It causes discrepancies in the acquired data from multiple experiments, especially between in analysis batches. Furthermore, it causes a critical problem when the statistical analysis is done because a lot of samples should be handled in the metabolome analysis. The purpose of this study was to develop a rapid and robust method of sample preparation for metabolomics using solid phase extraction (SPE). Extracted samples with acetonitrile were loaded on an ion-exchange cartridge SPE. Ion-exchange SPEs were used depending on what kind of chemical functional group the compounds have. The target compounds were retained in the SPE, then the cartridge was washed by acetonitrile for dehydration. Derivatization was done by methoxyamine/pridine and MSTFA which were directly added sequentially on the SPE. After derivatization, the object substances were eluted by acetone/n-hexane. The 10μL of derivatized compounds were injected in the GC-MS with a Large Volume Injection system equipped with a spiral shaped liner. A cation-exchange column, CXi, was used for amino acids which have cationic amino group, and an anion-exchange column, AXi, was applied for the compounds which have carbonyl and hydroxyl group, such as organic acids and sugars. Washing with acetonitrile after loading the target compounds enabled not only to eliminate other interferences but also to dehydrate at the same time. It took several hours to dry the samples in the conventional method, while dehydration was done in a few minutes using SPE. In this method, target metabolites which were retained in the SPEs by ion-exchange interaction were methoxyaminated by methoxyamine hydrochlolide in pyridine followed by trimethlysilylation effectively. Derivatized metabolites were changed into less-polar compounds and easily eluted using organic solvent, acetone/n-hexane. The total preparation time from sample loading on the column to derivatiation was within 10 minutes, and good reproducibility was obtained. By means of development of in-SPE derivarization method, we can achieve rapid and robust method of sample preparation for GC-MS metabolome analysis. Development of in-SPE derivarization method. Rapid and robust method of sample preparation for GC-MS metabolome analysis.

POSTER 409

Novel method for room temperature sample quenching and extraction of hydrophilic metabolites in cell culture using ionic liquids

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The preparation of samples for metabolomic analysis of cells grown in culture involves four distinct steps; metabolic quenching, cell lysis, extraction and sample concentration. Rapid metabolic quenching is important for maintaining an accurate snapshot of metabolic state without perturbations during sample manipulation and is most commonly accomplished through the use of low temperature (-40° to -60°C) solvents. However, care must be taken with quenching/extraction solvents to maintain quenched conditions and prevent leakage of intracellular metabolites. We have developed a room temperature method using fast filtration and ionic liquids that rapidly quenches metabolism, fully lyses a range of cell types and effectively extracts hydrophilic metabolites prior to LC-MS analysis and which is amenable to manual or automated operation. A sample from a cell suspension is initially filtered to remove cell growth media from the cell mass. The cells are washed and then rapidly quenched and lysed with the ionic liquid 1-Hexyl-3-methylimidazolium chloride (HMIM-Cl). The resulting mixture is subjected to solid phase extraction to separate lipids and proteins from hydrophilic metabolites. The eluate, containing aqueous ionic liquid, is then mixed with a water immiscible salt causing a metathesis reaction and ionic liquid extraction. The aqueous layer is then further extracted to remove traces of the ionic liquid. The resulting aqueous sample can be analyzed directly or concentrated by lyophilization. Initial tests were performed using a Luciferase Assay to demonstrate that ATP levels were constant in both yeast and E.coli samples after quenching with the ionic liquid. By contrast, ATP levels in control samples rapidly degraded. Microscopic evaluation of cells after ionic liquid addition showed complete disruption of a large proportion of both yeast and E. coli cells in contrast to cells subjected to more conventional published extraction procedures which appeared to remain largely intact suggesting that the use of ionic liquids could provide a more comprehensive disruption of cell walls and membranes during the quenching and extraction

processes. One advantage of the cationic ionic liquid used is its miscibility with the aqueous sample, but the appropriate anionic ionic liquid counter ion can be added to form an immiscible hydrophobic phase, which can easily be removed from the aqueous layer containing the hydrophilic analytes. Because residual amounts of ionic liquid may cause problems with analysis by mass spectrometry, we have also developed effective but simple steps to remove trace levels of the ionic liquid prior to LC-MS analysis. We performed LC-MS based analyses of a targeted set of 40 hydrophilic central metabolism components using a series of samples prepared from yeast and E. coli by the ionic liquid-based method described above and a conventional cold organic solvent quenching method. We will present data showing results from the ionic liquid based method are comparable to those obtained from conventional methods and a quantitative evaluation of speed and reproducibility of the two methods. Novel aspects include room temperature metabolite sample preparation, rapid metabolic quenching, effective lysis of cells, and ease of automation.

POSTER 410

DEVELOPMENT AND VALIDATION OF LIQUID CHROMATOGRAPHY-TANDEM MASS SPECTROMETRY METHOD FOR DETERMINATION OF MB12066 IN HUMAN PLASMA

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β-lapachone is a naturally produced compound in the bark of the South American Lapacho (Tabubuia avellanedae) tree. The compound is shown to possess antitumor activity against a variety of cancers. Currently, phase II clinical trials are being carried out on synthesized β-lapachone for its antitumor effect. MB12066 is a synthetic compound of β-lapachone. Although a number of studies have been conducted with regard to β-lapachone, relatively few publications describe quantitation methodologies for the analysis of β-lapachone or its metabolites. Furthermore, analytical methods for quantitating MB12066 using liquid chromatography-tandem mass spectrometry (LC-MS/MS) have not been reported yet. Therefore, the aim of this study is to establish a validation method for determination of MB12066 levels in human plasma by LC-MS/MS. The instruments, Agilent 1200 Series high-performance liquid chromatography(HPLC) system consisted of vacuum degasser, binary pump and autosampler with API 5000 triple quadrupole mass spectrometer equipped with electrospray ionization (ESI) source. The analytes were separated on a Zorbax XDB-C18 column (2.1 × 150 mm, 3.5 μm) employing an elution profile with a mobile phase of 0.1% formic acid in 5 mM ammonium acetate and acetonitrile, and a flow rate of 0.20 mL/min. Stock solutions (1 mg/mL) of MB12066 and MB12066-d6 (internal standard, IS) were prepared separately in acetonitrile. Working standard solutions of MB12066 and IS solution was serially diluted from stock solution. In this assay, acetonitrile was used for protein precipitation. Using LC-MS/MS in multiple reaction monitoring (MRM) scan mode, we are currently investigating linear response of MB12066 from 0.5 to 200 ng/mL. MB12066 and IS solutions were directly infused into MS with mobile phase (0.20 mL/min) and mass parameters were optimized to get maximum sensitivity for respective product ions. The precursor-to-product ion transitions at m/z 243.22 → 186.95 for MB12066 and m/z 249.26 → 186.97 for IS were used for quantification. As the compound is unstable under light irradiation, we are performing all experiments with amber tube to shade the light. We will further examine the additional test for accuracy, precision, specificity, recovery, and stability of the analytical method. We expect that LC-MS/MS method can be successfully validated for sensitive determination of MB12066 in human plasma and also can be acceptable for bioanalytical applications, according to the guidelines by the Ministry of Food & Drug Safety, Republic of Korea. Establishment for validation method can provide an ideal tool for high-throughput analysis of clinical samples and apply to pharmacokinetic studies.

POSTER 411

Simultaneous quantification of wide range of lipid mediators in human plasma using liquid chromatography electrospray ionization tandem mass spectrometry

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Primary lipid components of cellular membranes are used as an energy source as well as provide various important pivotal roles in host defense, acute inflammation, and neurotransmission, e.g., pharmacologic actions by lysophospholipid, and mitochondria-derived apoptosis induced by fatty acids. To understand the holistic view of these pathways, the development of simultaneous profiling of wide range of lipids are necessary. Here, we established a high-throughput and accurate analytical methodology using solid phase extraction and liquid chromatography-tandem mass spectrometry (LC-MS/MS) for profiling hundreds of lipids in human plasma samples. The analytes were purified by solid phase extraction (SPE). Separation of the analytes was achieved using mobile phase A consisted of acetonitrile/methanol/water at 20:20:60 (containing 5 mM ammonium formate), while mobile phase B consisted of isopropanol (containing 5 mM ammonium formate) at a flow rate of 0.3 mL/min on a Waters ACQUITY UPLC HSS T3 column. The analytes were quantified by LC-MS/MS using multiple reaction monitoring (MRM) mode. Solid phase extraction was employed in a processing protocol. Deproteinated plasma by methanol were loaded at SPE column (MonoSpinC18, GL Sciences Inc. Tokyo, Japan) to eliminate triglyceride (TG) and phospholipid phosphatidylcholine (PC) in the analytes. Totally, 227 kinds of lipids were detected among 402 multiple-reaction monitoring (MRM) transitions. These included bioactive lipid mediators, such as lysophospholipid, fatty acid, and sphingolipid. Among 1008 plasma samples obtained from Tsuruoka Metabolome Cohort Study, 129 compounds were consistently observed in 90% or more samples. The methods facilitated yielding the quantified catalogue of lipid pathways, which would contribute to the chronic disease-specific biomarkers. Simultaneous profiling of 402 lipids with a simple preparation protocol was developed.

POSTER 412

Novel HPLC-MS method for the detection of phosphorylated mono- and di-saccharides

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Carbohydrates are the most abundant group of organic compounds in nature. Phosphorylated carbohydrates are indispensable cogs in several key metabolic wheels for all forms of life. Evaluating the fluctuations of these compounds within various tissues and species is essential for the elucidation and understanding of how carbon pathways are regulated under environmental stress or disease. Due to the difficulty to differentiate the numerous isomers by mass spectrometry, a good separation upstream of MS detection is required. Here, a straightforward, liquid chromatography (LC) method coupled to mass spectrometry (MS) was developed to detect 10 phosphorylated sugars. Separation was optimized under HILIC conditions using a BEH amide column. HILIC is the most suitable LC approach for polar compounds analysed by MS. Methylphosphonic acid was added in the aqueous mobile phase to reduce tailing of phosphorylated groups, which are known to interact with stainless steel components. Alkaline pH conditions using triethylamine are also required to improve separations of the targeted compounds. The interactions between the ions present in the mobile phase and analytes are highlighted through the comparison of various eluents and conditions. The present method resolved eight phosphorylated sugars and thereby enables simultaneous detection and quantification of these compounds (R5P, G1P, M6P, G6P, S6P, L1P, T6P and F1,6bP). F1P and F6P were not resolved but quantification of fructose-phosphate is possible. The method was applied to real samples to compare metabolomic reactions of rice under submerged stress. Retention of phosphorylated sugars was due to a combination of HILIC mechanisms and ion pairing interactions.

POSTER 413

High-throughput Targeted Metabolomic Analysis of Oral Cancer Cells using Automated Column Switching and Ion Chromatography with HR/AM Mass Spectrometer

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Analysis of small polar metabolites is critical to understanding many metabolic disturbances, such as oral cancer. Recently it has been shown that ion chromatography (IC) when combined with high resolution accurate mass spectroscopy (HRAM) can provide superior separations and sensitivity for polar ionic species as compared to other separation methods. These results have been demonstrated using a capillary IC (45-min run) and replicated using a higher throughput method (20-min run) on an analytical IC system. To further improve the throughput we have developed a multiplexing system capable of alternating two columns and performing sequential injections of samples. A short gradient of 9-min run time has been achieved using the new platform and good resolution for isomeric metabolite was maintained. Polar metabolites from a reconstituted oral cancer cell lysates were injected and separated by a dual pump Dionex ICS-5000+ HPIC system and detected by a Q Exactive mass spectrometer in negative ionization mode, 140,000 resolution (FWHM at 200 amu), and 67–1000 m/z range. The column switching is realized using a 10-port valve programmed by Thermo Scientific Standard Instrument Integration (SII) software coupling Xcalibur and Chromeleon 7 platform. During separation mode, the KOH gradient is 25 to 99 mM KOH (0.2–7.9 min), and finished at 99 mM (7.9–9.1 min). During equilibration mode, the mobile phase is held at 99 mM KOH (0-4 min), and stepped down to 25 mM KOH (4min-end). It was previously reported that capillary ion chromatography (CapIC) offers at least 10 to 100-fold higher sensitivities than other methods using HILIC and RP chromatographies mainly due to the low background enabled by the suppressor technology. Additionally, 11 isomeric monophosphate sugars and nine isomeric diphosphate sugars in cell lysates were resolved by CapIC but not by HILIC or RP. These results were reproduced using an analytical IC system running a 20-min gradient, demonstrating the potential as a high throughput separation system. However, even shorter runs (e.g., < 10 min) were desired for targeted analysis of large sample sets. Here we further optimized the gradient to allow a 9-min separation with additional 5-min equilibration time. Six stable isotopic labeled (SIL) standards were well retained with reproducibility. Isomeric pairs like citrate and isocitrate; trans- and cis-aconitate were baseline resolved. Minimal resolution loss was observed for the isomeric mono- and di-phosphate sugars in the cell lysate samples. We then reconfigured the ICS-5000+ HPIC system to run column switching through an additional 10-port valve. The column switching (or multiplexing) was achieved by the new SII software which couples Xcalibur with IC operating system, Chromeleon 7. Both pumps had the same flow rate (0.38 mL/min) through separate eluent generator cartridges. When one column finishes the 9 min separation gradient delivered by pump 1, it switches to the equilibration gradient delivered by pump 2, and the eluent flows to waste. At the same time the second column switches inline to separation gradient delivered by pump 1. Such multiplexing was automated by programmed commands in the script; no other changes, such as additional pumps or suppressors are needed. In doing so, the total run time was further shortened but the actual equilibration time for each column was extended, resulting in further improved reproducibility. Automated column switching further increases the throughput of IC/MS in targeted metabolomics applications.

POSTER 414

Enhancing Ion Mobility-Mass Spectrometry Metabolomic Analyses with High Throughput Front End Separation Stages

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Metabolomic analyses of complex samples present numerous analytical challenges, such as isomeric indistinguishability and inadequate throughput of measurements. Ion mobility separations (IMS) minimize these limitations by providing high speed structure-informative analyses in conjunction with MS measurements. However, ionization suppression can occur due to the relatively large combined concentration of components in complex samples. In this presentation, we will report on the analysis of complex metabolomic samples utilizing RapidFire solid phase extraction (SPE) steps prior to IMS-MS measurements to reduce ionization suppression and detect metabolites with different properties. We also explored the use of field asymmetric waveform ion mobility spectrometry (FAIMS) prior to IMS-MS measurements to provide additional separation power for distinguishing metabolite isomers. The metabolomic separation capability of ion mobility mass spectrometry (IMS-MS) was demonstrated by analyzing various complex metabolomic samples from plasma to urine using an Agilent 6560 IMS-QTOF MS. Isomeric carbohydrate, fatty acid and lipid standards were also studied in depth in both positive and negative mode. A RapidFire 365 was coupled with the IMS-MS platform to study metabolites extracted from urine and plasma with multiple SPE cartridges, so that a wide range of metabolites with different properties could be analyzed rapidly while minimizing ionization suppression. An ultra-FAIMS (Owlstone, Cambridge, UK) was coupled with the IMS-MS measurements to understand the orthogonality of multidimensional separations and the effect of using different curtain gases. Carbohydrate isomeric standards from monosaccharides to hexasaccharides and fatty acids with different double bond positions and orientations were evaluated with IMS-MS. These isomeric compounds were well separated in both positive and negative mode, demonstrating that subtle differences in ring attachment and double bond positions could be distinguished due to structural variations. However, when more complex samples were analyzed with IMS-MS, ionization suppression was observed. A RapidFire SPE approach was utilized prior to the IMS-MS measurements to reduce ionization suppression. The RapidFire-IMS-MS measurements using multiple SPE cartridges enabled the detection of a wide range of polar and nonpolar metabolites simultaneously with high throughput (15 sec/cartridge). This approach was able to detect 10-fold more features compared to direct injection. The feature numbers obtained from RapidFire-IMS-MS analyses was comparable to LC-IMS-MS analyses, but with >100-fold greater speed. The ultra-FAIMS was also coupled with IMS-MS to study how multidimensional separations can potentially improve metabolite identification. Different types of curtain gases (100% nitrogen and helium/nitrogen mixture) were utilized with the helium/nitrogen mixture providing the best sensitivity based upon the number of features observed for a BSA tryptic digest. The ultra-FAIMS-IMS-MS analyses also showed the power of three dimensional separations by providing improved isomeric separations. The first evaluation of a RapidFire SPE pre-separation approach coupled with IMS-MS for high throughput metabolomic studies.

POSTER 415

Atypical myopathy - metabolite profiling of equine serum and urine

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Acquired equine multiple acyl-CoA dehydrogenase deficiency also known as atypical myopathy (AM), is a highly fatal muscle disease of grazing horses. This syndrome is accompanied by muscular weakness, stiffness, acute myonecrosis and myoglobinuria, which in at least 75% of cases leads to death within 72 h. It is probably caused by ingestion of Acer Pseudoplatanus seeds containing hypoglycin A, whose active metabolite, the methylenecyclopropylacetic acid, is responsible for an inhibition of some

dehydrogenases that using FAD as a cofactor (short and medium chain acyl-CoA dehydrogenases, isovaleryl-CoA dehydrogenase etc. [1, 2, 4]). The aim of this work was to perform whole metabolomic analysis in order to find other metabolic pathways which may be affected. Nine serum and six urine samples from horses with AM and twelve control samples were collected and then analyzed using high performance liquid chromatography (Ultimate 3000, Dionex) with aminopropyl column (Luna 3 µm NH2, 2 x 100 mm, Phenomenex) coupled to tandem mass spectrometry (QTRAP 5500, AB Sciex). The metabolites were detected by multiple reaction monitoring (MRM) in both positive and negative mode. Data were processed by unsupervised (Principal component analysis - PCA) and supervised (Orthogonal partial least squares discriminant analysis - OPLS-DA) multivariate analysis. The results of equine serum and urine show obvious separation of group with AM and control group. The differences were found not only in levels of glycine conjugates (isobutyrylglycine, hexanoylglycine, suberylglycine, phenylpropionylglycine), acylcarnitines (C2 - C20), which well corresponds with previously published results [2, 3], but also in purine metabolites (inosine, hypoxanthine, adenine, adenosine) and some organic and amino acids (aspartate, cysteine, tryptophan, homoarginine, betaine) etc. suggesting that AM affects the other metabolic pathways. The work was supported by grants Czech Science Foundation Grant I 1910-N26, LF UP 2014-011, IGA VFU Brno, No. 86/2014/FVL and CZ.1.07/2.3.00/30.0004. The infrastructural part of this project (Institute of Molecular and Translational Medicine) was supported from NPU I (LO1304). [1] Votion D-M. et al., Equine Vet J. 2014, 46(2), 146-149. [2] Valberg S. J. et al., Equine Vet J. 2013, 45(4), 419-426. [3] Westermann C. M. et al., Neuromuscul Disord. 2008, 18(5), 355-364. [4] Unger L. et al., J Vet Intern Med 2014, 28, 1289-1293. complex metabolite profiling of horses with Atypical myopathy

POSTER 416

Development of a high-throughput and untargeted UPLC-MS/MS lipidomic platform for identifying predictive disease markers in transition dairy cows

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Untargeted lipid profiling using UPLC-MS/MS is a powerful technique for elucidation of complex lipid classes in biological samples. The most popular lipid sample preparation methods require toxic solvents, or are relatively time consuming. It is desirable to have a global sample preparation method that is safe, simple for fast extraction and purification of different lipid classes in complex biological samples. In this study, we have developed and applied a high-throughput sample preparation technique for untargeted LC-MS lipidomics for the identification of predictive serum lipid biomarkers of disease risk at early stage in transition dairy cows. Our technique is less-toxic and much simpler than the state-of-the-art methods, and comprehensively covers the predominant lipid classes. In order to identify predictive lipid biomarkers, we collected from 161 multiparous cows serum samples 3, 2, and 1 week before calving and at calving. For this nested case-control study, we selected serum samples of cows that either developed after calving mastitis (n=8), or remained healthy (n=9). The serum samples were separated by centrifugation, and 3 volume of chilled isopropyl alcohol added. Samples were vortex-mixed for 30 sec, and stored at -20 °C overnight. On the following day, samples were centrifuged at 14,000 x g for 15 min. The supernatant was transferred to fresh tubes for UPLC-MSE analysis. Data analysis was performed with XCMS R package, MetaboAnalyst, GEDI (Gene Expression Dynamics Inspector), and online databases (METLIN, HMDB, and Lipid MAPS). Lipid profiles were acquired in both positive and negative electrospray ionization modes using high resolution q-TOF- mass spectrometry (Waters Synapt G2) coupled to ultra-performance liquid chromatography (Waters Acquity UPLC I class). Mass-spectral datasets were evaluated using multivariate statistical analysis. The lipid patterns were clearly distinguished between the healthy and mastitis groups at each time point that appeared as distinctive clusters in PLS-DA plots. The analysis of untargeted lipidomics data leads to detection of 160 lipids in positive mode, and 350 total lipids in negative mode in a 15 min acquisition. Using isopropylalcohol (IPA) for lipid extraction was a simple, and safe sample preparation procedure which resulted in coverage of a broad range of different classes of lipids. These included lysophospholipids, phospholipids, sphingolipids, sterols (cholesterols, cholesteryl esters, and bile acids), non-esterified fatty acids (NEFAs), prenol lipids, as well as low abundant lipids in cows' serum such as glycerol lipids. The coefficient of variation of identified lipid sub-classes, and internal

standards were less than 10%, demonstrating excellent reducibility of the use of IPA for lipid extraction. We applied self-organizing maps algorithm on the average intensity level of significant features at different time points. Interestingly, we observed a trend in clustering of ions before calving, and at calving, which represent a dynamic change in the intensity levels of lipid profiles remarkably in triacylglycerols (TAGs), lysoPCs and lysoPEs, and NEFAs. The intensity level of different subclasses of TAGs were significantly changed in mastitis ($p<=0.02$) at 3 weeks before calving. These finding suggest changes in serum TAG levels might be an early indicator for the development of mastitis in dairy cows. First untargeted comprehensive lipidomics analysis using IPA for sample preparation of cow serum.

POSTER 417

Metabolomics identifies potential screening and diagnostic serum biomarkers of laminitis in transition dairy cows

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Laminitis is one of the most common periparturient diseases of dairy cows. It is costly in terms of lowering reproductive performance, decreasing milk yield, and treatment costs. Laminitis may go through a subclinical phase in its early stages and is often difficult to detect before clinical signs (i.e. lameness and laminitis-related lesions) appear, and by the time it is detected it is often too late to treat. Improved methods for detecting the onset and evolution of laminitis would help manage the disease and facilitate early intervention. The objective of this study was to evaluate alterations of metabolites in the serum of dairy cows with laminitis during the transition period and identifying screening blood biomarkers for the disease. Blood samples were collected from 100 dairy cows and 6 cows that developed laminitis and 6 healthy cows were selected for intensive analyses. Blood samples were obtained at 5 time points during the transition period (-8, -4, disease diagnosis, +4 and +8 wks relative to parturition). Serum metabolic profiles between the two groups of cows were compared by a targeted quantitative metabolomics approach using a combination of direct injection and tandem mass spectrometry (DI-MS/MS) with a reverse-phase liquid chromatography and tandem mass spectrometry (LC-MS/MS) kit. Univariate and multivariate data analyses (principle component analysis (PCA) and partial least square discriminant analysis (PLS-DA)) were applied at each time point to examine alterations of serum metabolites throughout the progress of the disease. A total of 128 serum metabolites including amino acids, glycerophospholipids, sphingolipids, acylcarnitines, and hexose were identified and quantified. Specifically, 98 metabolites were quantified by DI-MS/MS and 30 metabolites were quantified by LC-MS/MS. Results from univariate analysis indicated that cows with laminitis experienced elevated concentrations of 25, 56, 74, 35 and 28 metabolites in the serum of cows more than 4-8 wks before development of laminitis as well as during the week of diagnosis of the disease, and 4-8 wks after laminitis diagnosed. The identified metabolites are involved in various metabolic pathways associated with biotin metabolism, amino acid pathways (including valine, leucine, isoleucine, histidine, arginine, proline), nitrogen metabolism, and bile acid biosynthesis, all of which might play specific roles in the etiopathogenesis of laminitis. PCA and PLS-DA analysis also showed separate clusters between the healthy and laminitis cows at all 5 time points on the basis of the measured serum metabolites. The data suggest that alterations in blood metabolites of several metabolic pathways precede development of laminitis but also they accompany clinical symptoms of laminitis. Variable importance projection (VIP) data indicated that five metabolites including lysine, leucine, isoleucine, PCaaC30:2, and SM(OH)C24:1 were the most influential factors to differentiate laminitis-affected cows with the healthy ones during the whole experimental period. Receiver operator characteristic (ROC) curve analysis needs to be conducted to build optimal biomarker models with high sensitivity and specificity. In conclusion, data presented here

indicate that development of laminitis is preceded with perturbation of several metabolic pathways and give insight into the etiopathology of laminitis while suggesting promising areas of focus for future studies. Moreover, results also demonstrate that the metabolomics approach can generate novel hypotheses regarding disease mechanisms of laminitis with the potential to identify novel serum metabolites as screening and diagnostic biomarkers in transition dairy cows. This is the first report showing that blood metabolic alterations precede more than 8 wks of laminitis in dairy cows.

POSTER 418

Metabolomics reveals pathways and biomarkers perturbed before clinical appearance of milk fever in transition dairy cows

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Milk fever (MF), or parturient paresis, is a common postparturient disease of transition dairy cows triggered by an imbalance of calcium metabolism around calving. Cows affected by MF are at greater risk for developing reproductive disorders and more susceptible to other metabolic and infectious diseases such as displaced abomasum, retained placenta, ketosis, uterine prolapse, and mastitis. Milk fever has been studied for more than 200 years but the underlying etiology still remains unclear. The objective of this study was to investigate alterations of serum metabolites related to major metabolic pathways in cows with MF and to identify the mechanism underlying MF and the potential for identifying blood screening and diagnostic biomarkers associated with the onset and progress of the disease. A total of 100 cows were included in the experiment starting at 8 wks prior to parturition. Blood samples were collected at -8, -4 before parturition and during disease diagnosis week as well as at +4, and +8 wks postpartum. Six cows that developed MF and 6 healthy cows were selected for intensive analyses using LC-MS/MS and DI-MS/MS by a Hybrid Triple Quadrupole/Linear Ion trap mass spectrometer. Differential metabolites were identified with Wilcoxon-Mann-Whitney (rank sum) test ($P<0.05$), principle component analysis (PCA), and partial least square discriminant analysis (PLS-DA) at 5 time points, respectively. Metabolic pathway analysis for the significant metabolites was applied to interpret pathophysiological changes in MF-affected cows starting at 8 wks prior to occurrence of clinical MF. A total of 128 metabolites belonging to 5 distinct analyte groups including amino acids (29), acylcarnitines (7), glycerophospholipids (77), hexose (1), and sphingolipids (14) were identified and quantified in the healthy and MF cows. Based on the univariate analysis 27, 31, and 16 serum metabolites including sarcosine, carnosine, creatinine, alanine, and glutamine were elevated in cows with MF during -8, -4, and during the week of diagnosis of disease, respectively. Metabolomics data also revealed a distinct metabolic signature for cows affected by MF including metabolites from tricarboxylic acid (TCA) cycle, arginine, proline, glycine, serine and threonine metabolism as well as aminoacyl-tRNA biosynthesis, phenylalanine, tyrosine, and tryptophan biosynthesis. Both PCA and PLS-DA analyses indicated 2 clearly separated clusters of metabolites at all 5 time points analyzed. Furthermore, 5 metabolites (i.e., lysine, isoleucine, leucine, PC aa C30:2, and sarcosine) accounted for most of the observed separation between the two groups of cows, based on their VIP values (variable projection importance plot). Receiver operating characteristic (ROC) curve analysis needs to be done for the preliminary evaluation of the identified biomarkers. In conclusion, alterations of serum metabolites and disturbed metabolic pathways in MF cows begin more than 8 wks before parturition and even earlier than the occurrence of the disease itself, highlighting the importance of metabolomics approach for development of early screening biomarkers of the disease. Our data also suggest that metabolomics is a reliable approach to further increase our understanding of the causal agent(s) and pathogenesis of MF, and help identify early diagnostic and prognostic biomarkers for MF in transition dairy cows. Metabolomics identified multiple alterations in the blood of dairy cows prior to development of MF that can serve as biomarkers.

POSTER 419

Metabolic fingerprinting to reveal a novel biomarker for prednisolone treatment in cattle

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Prolonged exposure to synthetic glucocorticoids, like prednisolone, results in growth-promoting side effects. As a result thereof synthetic glucocorticoids may be fraudulently administered to meat-producing animals. In order to protect consumers against potential harmful residues, the use of synthetic glucocorticoids in livestock has been strictly regulated in the European Union. However, a higher frequency of non-compliant bovine urine samples for prednisolone has been noticed, which could not be directly related to fraudulent use. Questions have risen about the origin of this compound. At present, no decisive strategy has been established to discriminate between endogenous formation and exogenous administration of prednisolone. Here, a metabolomics approach was used to reveal and (tentatively) identify biomarkers in bovine urine to prove exogenous prednisolone treatment. An in vivo study was conducted with 12 adult cows that underwent a growth promoting treatment (low dosage and long-term) and a subsequent therapeutic treatment (high dosage and short-term) with prednisolone. The collected urine samples were analyzed by both full-scan UHPLC-Orbitrap MS and UHPLC-QqToF-MS to acquire the specific metabolic fingerprints corresponding to the different prednisolone treatments. Peak list generation was performed using either SieveTM 2.1 (Thermo Fisher Scientific) or MarkerviewTM 1.2.1.1 (AB SCIEX) software to respectively process Orbitrap MS and QqToF-MS data files. Afterwards multivariate analysis by means of Orthogonal-Partial Least Squares-Discriminant Analysis (OPLS-DA), using SIMCATM 13 software (Umetrics), was employed to search for potential biomarkers linked to exogenous prednisolone administration. The potential biomarkers were identified using an in silico based strategy. After peak list generation, the acquired data matrices were normalized by means of specific gravity and reorganized into a control group (Control, n = 120) and a treatment group (Treatment, n = 216), which comprised all urine samples that were either collected prior to or during prednisolone treatment. Multivariate statistical analysis was performed, by means of OPLS-DA, in order to reveal significant differences between the metabolic fingerprints, associated with the various treatments. Based on the S-plot and VIPOSTER score, four potential differentiating metabolites were revealed. None of these compounds were present in bovine urine containing endogenous prednisolone, of which the formation was induced by adrenocorticotrophic hormone (ACTH) administration. This indicated good selectivity of the metabolites. Besides, biological relevance of these ions was determined by means of sensitivity and specificity based on their occurrence in the treatment and control samples. These showed that only one metabolite was highly suitable as a potential biomarker during growth-promoting and therapeutic prednisolone treatment, being reflected by the 93.4% sensitivity and 96.3% specificity. Besides, the urinary profiles of the metabolites were considered since insights in the metabolites' kinetics could be of special interest to extend the detection period of illegal prednisolone abuse, as an additional criterion for suitability of the potential biomarkers. Therefore, the pharmacokinetics of urinary excretion of the revealed compounds was monitored after a single therapeutic dose of prednisolone. The most potent compound could be detected up to four days after a single prednisolone administration. Based on accurate mass, isotope pattern, and MS/MS spectra, the four newly defined biomarkers were putatively annotated and one metabolite was suggested as an actionable biomarker for exogenous prednisolone administration. Four biomarkers with the ability to discriminate between endogenous formation and exogenous administration of prednisolone were identified in bovine urine.

POSTER 420

Molecular characterization of Bruck Syndrome shows differential expression profile during in vitro osteoblastic differentiation of bone marrow mesenchymal stromal cells

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Osteogenesis Imperfecta (OI) is a heterogeneous group of inherited disorders characterized by increased bone fragility, with clinical severity ranging from mild to lethal. The majority of OI cases are caused by mutations in COL1A1 or COL1A2. A further recessively-inherited OI-like phenotype is Bruck Syndrome (BS), in which bone fragility is associated with the unusual finding of pterygia and contractures of the large joints. Notably, several studies have failed to show any abnormality in the biosynthesis of collagen 1. Recently, evidence was obtained for a specific defect of procollagen telopeptide lysine hydroxylation in BS and mutations in the gene PLOD2 have been identified. However, there is little information about PLOD2 and genes expressed in undifferentiated and differentiated BMSCs from BS patients. Mononuclear cells obtained from the bone marrow of BS, OI patients and healthy donors were cultured and osteogenic differentiation was induced. Total RNA was isolated and concentration was determined by photometric measurement. High Capacity cDNA Reverse Transcription Kit was used to synthesize cDNA of 1 µg RNA. Genomic DNA was obtained from undifferentiated BMSCs in the third passage with Wizard Genomic DNA Purification Kit (Promega). DNA sequencing of PCR-amplified COL1A1, COL1A2, and PLOD2 gene fragments covering the entire coding region and intron/exon boundaries was carried out using an ABI PRISM 3130 automated sequencer and Big Dye Terminator Sequencing protocol. RT-PCR assays were performed to detect the expression levels of osteoblast specific marker genes BGLAP, COL1A1, MSX2, SPARC, and VDR. All OI patients showed the characterized mutations in COL1A1 or COL1A2 genes. Those mutations lead to amino acid substitutions that are probably responsible for the observed phenotype. In DNA from BS patient, two COL1A1 and one COL1A2 mutations were found: one transition T>C at position 3,459 and another C>T at position 3,897 of COL1A1. In COL1A2, a T>C transition at position 87 was found. Surprisingly, none of the mutations found in BS patient resulted in protein sequence alterations. No mutations were found in PLOD2 gene. The osteogenesis was monitored by microscopy during the whole in vitro differentiation period. The first signs of calcification appeared as black regions within the cell monolayer in approximately ten days. The maximum extracellular matrix calcification was observed after 21 days of differentiation. Gene expression of osteoblast specific markers BGLAP, COL1A1, MSX2, SPARC and VDR was evaluated by Real Time RT-PCR during differentiation into osteoblasts and the results showed similar patterns of osteoblast markers expression between BS and healthy controls. On the other hand, when compared with OI patients, the expression pattern of these genes was different. The present study shows for the first time that genes involved in osteogenesis are differentially expressed between BS and OI patients. We suggest that gene expression profiles during MSC differentiation into osteoblast are distinct in BS and OI patients.

POSTER 421

Human urine metabolome of irritable bowel syndrome patients: insights into disease pathophysiology

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Irritable bowel syndrome (IBS) is a chronic gastrointestinal disorder characterized by abdominal pain and

altered bowel habits, frequently accompanied by anxiety and depression. This disorder affects 15-20% of the world population with an especially high incidence reported in Canada. Its diagnosis is largely based on symptoms as the etiology of IBS remains poorly understood . In this study, urinary metabolites of IBS patients were analyzed using multi-segment injection-capillary electrophoresis-mass spectrometry (MSI-CE-MS) and compared with that of control (non-IBS) individuals. Since gut bacteria are implicated in IBS pathophysiology, urinary metabolites differentially expressed in IBS patients were investigated to elucidate specific biochemical signatures associated with gut microbiota, diet and host co-metabolism and their clinical history based on Rome III diagnostic criteria. Single-spot urine samples of 43 IBS patients with age ranging from 19 to 70 were collected during a morning clinic visit. Age and gender matched control urines were also collected in the morning. All urine samples were diluted with sodium azide after collection and stored under -80^{o}C prior to analysis. Upon thawing and diluting with internal standards, randomized urine samples were analyzed by MSI-CE-MS under positive and negative ion modes for characterization of cationic and anionic metabolites, respectively. A pooled urine sample was also included within each analysis as a quality control to monitor long-term system stability. Over 130 anionic and cationic metabolites were consistently measured in pooled urine specimens after rigorous data filtering to remove chemical and biochemical noise, including background signals, co-migrating adducts and in-source fragment ions. Known polar/ionic metabolites analyzed in urine included compounds related to activities of gut microflora, such as cresol sulfate, indoxyl sulfate and hippuric acid, as well as compounds associated with diet and lifestyle, such as trigonelline, acetaminophen sulfate and ethylsulfate. Reproducible urinary metabolites were selected for univariate and multivariate statistical analysis based on the two inclusion criteria, namely signals were detected in 75% of total samples with good precision (< 30% relative standard deviation) for quality control samples. To account for the large intrinsic biological variance and hydration status, data were analyzed with creatinine normalization using complementary data transformation methods. Differences in the level of excreted metabolites between control and IBS groups were quantified with a specific focus on compounds associated with serotonin/kynurenine pathway based on their significant roles in the gastrointestinal tract motility and as neurotransmitters associated with the brain-gut axis. Additionally, differences in excreted indole metabolites and short-chain organic acids were investigated given their putative roles as biochemical signalling molecules among intestinal microbiota. Physiological data derived from individual IBS patients, such as medical history and severity of symptoms were incorporated into the analysis to elucidate the pathophysiology of IBS and characterize disease heterogeneity as required for better treatment decisions in the clinic. Novel Aspects: Microbial and dietary metabolites in human urine are analyzed to investigate the pathophysiology and heterogeneity of IBS. Microbial and dietary metabolites in human urine are analyzed to investigate the pathophysiology and heterogeneity of IBS

POSTER 422

Nitaxozanide as repositioning drug in the treatment of leishmaniasis: a metabolomic study by GC-MS

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Leishmaniasis is a neglected disease affecting more than 12 million people in 88 countries worldwide. Treatment is limited to highly toxic drugs and some of them are not effective due to parasite resistance. An alternative strategy for the treatment of several infectious diseases is the use of repositioning drugs. In this work, a metabolic fingerprinting approach using GC-MS was applied to Leishmania infantum promastigotes, wild type and treated with nitazoxanide (IC50 42.71 mg/mL). Nitazoxanide has been traditionally used to treat some protozoa and helminths, but presented good anti-Leishmania activity. The action of this drug has been associated with oxidative stress metabolism and by GC-MS it is possible to assess metabolites such as amino acids and derivatives, fatty acids, and others directly in the parasite, that can help to understand the metabolic pathways altered by the treatment. The use of repositioning drugs is therefore presented here as a promising strategy in the treatment of leishmaniasis, since they

have been already thoroughly tested with respect to clinical safety, and toxicity. Moreover, possible side effects have been established, reducing pre-clinical costs, and making the drugs available to patients more quickly.

POSTER 423

Integrative 'omics of polymicrobial model cultures from Cystic Fibrosis derived strains

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Patients with Cystic Fibrosis (CF) are prone to polymicrobial colonization of the airways due to the presence of thick, immovable mucus. Current clinical culturing methods focus on individual opportunistic pathogens, cultured independently. Interactions between microbes are often mediated through small molecule metabolites and profoundly influence microbial physiology, including growth and antibiotic resistance profiles. In previous studies we have found that microbial fermentation products such as 2,3-butanediol, likely produced by Streptococcus spp., are more abundant during periods of worsened symptoms, and decrease with antibiotic treatment. An important goal is to identify microbial metabolite biomarkers of infection, and understand their impact on microbial physiology. We are using a multi-omics approach to characterize the interaction between clinical isolates of three Pseudomonas aeruginosa strains, Streptococcus salivarius, and Streptococcus pneumonia, and comparing what we learn with previous studies of metagenomes and metabolomes from sputum samples donated by the same patient. Illumina sequencing of the microbial genomes enables comparative genomic analysis, and comparison with metabolomic and transcriptomic data. CF clinical isolates are grown in lab media and also Artificial Sputum Media (Quinn and Whiteson et al ISME doi:10.1038/ismej.2014.234), to replicate the conditions in the lung as well as possible. Metabolite profiling is being conducted with both GC-MS at the UC Davis West Coast Metabolomics Core Facility, and by LC-MS/MS. Preliminary evidence suggests microbial fermentation products such as 2,3-butanedione may be important indicators of periods of worsened symptoms in clinical studies. They may also increase microbial virulence and trigger the host immune response. Metabolites that are uniquely produced by particular microbes are good markers for the active microbes in a disease state. Some metabolites (e.g. fermentation products) are only produced under certain conditions, and thus provide insight into the local environment (e.g. pH, oxygen and nutrients). Fermentation products in the airways are exclusively produced by microbes (as opposed to human cells), and they are promising biomarkers because they are detectable and accessible. In vitro co-culture systems are manipulatable, and can be compared to human clinical samples. Molecules present in cultured samples that are found to be more abundant in the moments of worsened symptoms are more likely to have microbial origin. In addition, molecules that are found to be uniquely increased or decreased in co-cultures of Pseudomonas and Streptococcus relative to the individual cultures can be attributed to their interaction. Cultures are conducted with a gradient of complexity, including 1) individual cultures of Pseudomonas and Streptococcus strains, 2) individual cultures grown in the presence of molecules known to be more abundant during moments of increased symptoms, including 2,3-butanediol, 3) swapping the supernatants from cultures grown independently, and 4) co-cultures of each Pseudomonas strain with each Streptococcus strain. The metabolite profiles of individual cultures of Pseudomonas and Streptococcus are unique, and also different from their co-culture. Molecules that are noticeably increased in the co-culture of a more virulent P. aeruginosa strain with S. salivarius include trehalose, ribose and thymidine. In preliminary experiments, S. salivarius produces 2,3-butanediol in these conditions, and in much greater abundance individually than in the co-culture. Including neighboring microbes and physiologically relevant conditions will improve our ability to diagnose and treat polymicrobial infection.

POSTER 424

Molecular Networking Based Metabolomics Identifies Microbial and Host Changes During Cystic Fibrosis Exacerbations

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Cystic Fibrosis is a genetic disease that results in the buildup of thick mucus in the lungs susceptible to colonization by bacterial, fungal, and viral pathogens. CF lungs harbor a complex microbial community that changes and adapts through the patient's lifetime. Patients intermittently experience cystic fibrosis pulmonary exacerbations (CFPE), events characterized by increased symptoms requiring aggressive antibiotic treatment. CFPEs are associated with poor clinical outcomes and significantly decrease patient quality of life. The causes of CFPE are poorly understood, but are believed to involve changes in the microbial community and host response. LC-MS/MS based metabolomics was employed to investigate chemical changes in mucus samples through CFPE events. Eight patients were analyzed for a total of 43 mucus samples. These samples represented one of four disease states: stable, exacerbation, treatment, or recovery. One patient donated 16 samples total, including those collected at daily time points, enabling a detailed longitudinal study. Bacterial 16S rDNA sequencing was generated on these daily samples to compare to metabolome changes. The molecular networking and random forest statistical algorithms were applied to the data as an untargeted approach to the analysis. The novel mass spectrometry MS/MS database GnPS was used to automatically annotate molecules. Clustering of the metabolome similarity using random forests demonstrated that the samples clustered better by patient than disease state. This indicated that the mucus chemistry of individual patients was very different from one another. However, monitoring a single patient with daily sampling showed large changes in the metabolome through a single CFPE. At the onset of this CFPE the metabolome was markedly different than at times of stability or treatment. Antibiotics had a large effect on the metabolome of these samples, where large fluctuations in the metabolome data were observed. These fluctuations were associated with increases and decreases in the abundance of host sphingolipids, particularly ceramide, sphingomyelin, and sphingosine. Comparison of microbial 16S rDNA sequencing on the same daily samples revealed molecular and bacterial communities associated with the CFPE. At exacerbation anaerobic bacteria were most abundant with a unique community of molecules correlated with their abundance. Pseudomonas aeruginosa and Stenotrophomonas maltophilia were the most prevalent during treatment and also had a uniquely associated chemical community. There were no molecules co-correlated between either the exacerbation or treatment microbial communities, demonstrating their exclusivity. This study showed that CF patients have a very personalized chemical community within their lung mucus, but at exacerbation, this community is drastically altered. These alterations are associated with changes in the lung microbiome, indicating that host and microbial chemistry is linked through CFPE events. This study is one of the first to link microbiome and metabolome data in a medically relevant context.

POSTER 425

A multi-platform metabolomics approach identifies novel biomarkers associated with bacterial diversity in the human vagina

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Bacterial vaginosis (BV) is the most common vaginal condition, characterized by a decrease in Lactobacillus species with a concurrent increase in bacterial diversity. BV increases transmission of HIV, enhances the risk of preterm labour, and its associated malodor impacts the quality of life for many women. Clinical diagnosis primarily relies on microscopy to presumptively detect a loss of lactobacilli and acquisition of anaerobes. This diagnostic does not reflect the microbiota composition accurately as lactobacilli can assume different morphotypes, and assigning BV associated morphotypes to specific

organisms is challenging. We utilized an untargeted multiplatform metabolomics approach (GC-MS and LC-MS), combined with 16S rRNA gene sequencing to profile the vaginal metabolome and microbiota of 131 pregnant and non-pregnant Rwandan women. Using a series of partial least squares (PLS) regressions we determined the variable that what most associated with changes in the vaginal metabolome. Correlations between the microbiota and metabolome were used to identify potential sources of metabolites of interest. Metabolic products in the vagina were strongly associated with bacterial diversity, and the metabolome did not differ between pregnant and non-pregnant women. We identified two novel biomarkers associated with diversity and clinical BV that were highly specific for the condition (AUC=0.99). These biomarkers were independent of pregnancy status, and were validated in a blinded replication cohort from Tanzania (n=45), in which we predicted clinical BV with 91% accuracy. Additionally, we identified the same biomarkers in Canadian women (n=35), demonstrating they are globally applicable for the diagnosis of BV. Correlations between the metabolome and microbiota identified Gardnerella vaginalis as a putative producer of one of these biomarkers, and we demonstrate production by this species in vitro. We have identified novel biomarkers for BV and are the first to profile the vaginal metabolome of pregnant women.

POSTER 426

A metabolomics insight into immunotoxic effects of natural products

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A variety of xenobiotics, e.g. from nutritional or environmental sources, have been shown to influence the immune system. To date immunotoxicity is mainly investigated by in vivo experiments using laboratory animals and thus, with a limited predictability for human health. In this in vitro study three human cells lines are in use for the treatment with immune modulating compounds representing different parts of the immune system: Jurkat (T cells), RPMI1788 (B cells) and THPOSTER 1 (monocytes). With the combination of cell biological assays, OMICS-technologies, systems biology integration, and modelling of accumulated data we want to assess the immunotoxic potential of compounds and get an insight into the molecular mechanisms for a better prediction of immunotoxicity and a reduction of animal experiments. Data analysis was realized using a platform of three different parts. The extracellular metabolome including medium constituents and metabolites secreted during growth of the cells was analysed by 1H-NMR spectroscopy. 2D-NMR experiments offer possibilities for identification of unknown metabolites. Amino acids and polar intracellular metabolites of the central carbon metabolism, including glycolysis and TCA cycle were investigated after derivatization using GC-MS methods. Nucleotides and other highly charged compounds were identified and quantified using an ion-pairing HPLC method connected to ESI-TOF-MS detection. A protocol was developed for an efficient and reliable sampling procedure to analyse intracellular metabolites of Jurkat cells. Many medium constituents are also metabolites of the intracellular metabolome. Hence separation of the cells from the complex RPMI medium was a very important step. Only low amounts of metabolites could be extracted from cells obtained by filtration compared to sampling by centrifugation. Therefore the centrifugation approach was optimized concerning cell number, quenching of the metabolism, cell loss and washing steps. For extraction of intracellular metabolites three different methods were compared. Optimized conditions included the stepwise use of methanol, water and chloroform (5/5/1, v/v/v) as extraction solvents and lyophylization of the extracts. The sampling protocol was used for generating intracellular metabolome samples from Jurkat cells treated with four immune modulating substances at concentrations of IC10 and untreated cells (as control). Deoxynivalenol, cannabidiol and vincristine were used for treatment as immunosuppressive agents. For tulipalin A immunostimulating effects are described. By investigating the changes of metabolite concentrations of the central carbon and nitrogen pathways, questions on the role of these metabolites during immunotoxic stress can be addressed in detail. Most differences in the concentrations of the

extracellular metabolites between treated and untreated cells were observed for cannabidiol and deoxynivalenol. While a lower cell number was reached after the incubation with cannabidiol for 72 h glucose uptake was increased. The concentrations of serine, valine, glycine and lysine were found to be 2 to 3 fold higher compared to controls contrary to the concentration of extracellular pyruvate which was only 26 % of the control level. For treatment with deoxynivalenol a reduced uptake of glucose was found and the extracellular pyruvate level was 1.6 fold higher than the level of the untreated control. For all differences the statistical significance was determined ($P < 0.05$, Student's t-test). Metabolomic studies of the human derived Jurkat T cell line are used to screen for mechanisms of immunotoxicity.

POSTER 427

13C isotope tracing reveals hypoxia as a novel regulator of adipocyte branched chain amino acid catabolism

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Plasma branched chain amino acid levels are elevated in obese and diabetic patients and dysregulated adipose tissue metabolism is proposed to contribute this. Hypoxia may play a central role in obesity and diabetes-induced adipose tissue dysfunction and the prevalence of obstructive sleep apnea exceeds 30% in obese subjects. Adherence to OSA treatment correlates with a decrease in fasting blood glucose in obese OSA patients indicating that hypoxia may play a central role in altered adipocyte metabolism and thus contribute to impaired whole body BCAA metabolism. Here, we apply 13C isotope tracing to both human and 3t3l1 adipocytes to probe relative utilization of the primary carbon sources of adipocytes in hypoxia for the first time. Day7 post induction 3T3L1 adipocytes were cultured in the presence of either 13C Glucose, 13C Glutamine, 13C Leucine, 13C Isoleucine or 13C valine supplemented DMEM for 24 hours in 21% or 1% oxygen. Polar and lipid metabolites were extracted using methanol/chloroform and analysed by GCMS. Metabolite integration and correction for 13C natural carbon abundance was carried out using INCA. Spectral analysis was performed to determine the % newly synthesized lipids and % contribution of different carbon sources to de-novo lipogenesis. Human adipocytes were derived from subcutaneous or omental adipose depots and primary brown adipose tissue was derived from C57BL6 mice. In vivo oxygen tension was measured in C57BL6 mice using a needle polarographic oxygen electrode. We have found in contrast to proliferating cells that primarily use glucose and glutamine for AcCoA generation, differentiated adipocytes increase flux through the BCAA catabolic pathway such that leucine and isoleucine accounted for as much as 30% of lipogenic AcCoA pools. When glucose and amino acids were present at physiological levels BCAA catabolism was fueled by protein turnover. Exposure of 3T3L1 adipocytes to hypoxia decreased glucose contribution to citrate and de novo lipogenesis by 13% and 10% respectively and glutamine utilization for de novo lipogenesis increased from 7% in normoxia to over 30% in hypoxia. However, in contrast to the more subtle effects of hypoxia on glucose entry into the TCA cycle, BCAA catabolism was dramatically decreased in hypoxia with leucine contribution to citrate decreasing by 90% relative to normoxia and incorporation into lipids was undetectable. A similar decrease in BCAA catabolism was found in hypoxia treated human adipocytes. In addition to the acute effects of hypoxia on BCAA catabolism, following reoxygenation, BCAA catabolism was still significantly impaired. However, both glucose and glutamine utilization returned to a normoxic phenotype upon reoxygenation. Analysis of BCKDH and phosphorylated BCKDH levels revealed that an overall decrease in BCKDH expression was driving the decrease in BCAA catabolism rather than an increase in BCKDH phosphorylation. Brown adipose tissue was also found to catabolize BCAAs to a similar extent as WAT and hypoxia induced a similar to decrease in catabolism, however BCAA catabolism was fully restored upon reoxygenation in this cell type. To investigate whether adipose tissue was particularly susceptible to low oxygen levels during intermittent hypoxia relative to other tissues, we measured in vivo tissue oxygen tension during IH treatment in mice. Adipose tissue became significantly more hypoxic and oxygen levels recovered slower than muscle indicating that adipose tissue is more susceptible to hypoxic insults. This work identifies that hypoxia may be a novel driver of dysregulated BCAA catabolism in obesity.

POSTER 428

GC-MS based untargeted metabolomic analysis of kidney and liver tissue from the Lewis Polycystic Kidney (LPK) rat

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Polycystic kidney diseases are inherited and lead to end-stage kidney disease. The most common form is autosomal dominant PKD, but there are others such as the nephronophthisis (NPHP) group of ciliopathies. There is no cure for PKDs and current treatment options are limited to renal replacement therapy and transplantation. A better understanding of the pathogenesis of PKDs is needed for the development of new, less invasive treatments. The LPK rat phenotype has been characterized and classified as a model of NPHP9 caused by mutation of the NEK8 gene. The aim of this study was to use a GC-MS based untargeted metabolomics approach to determine key biochemical changes in LPK rat tissues. Ten 16-week old LPK and 11 Lewis age- and sex-matched control animals were used. Animals were anaesthetised and euthanized by exsanguination cardiac arrest. Organs were harvested, washed with physiological saline, frozen on dry ice, and stored (-80°C). Kidney and liver organs were freeze-dried whole, ground using a mortar and pestle and weighed into lysis tubes. Metabolites were extracted with methanol and water containing 13C6-sorbitol (IS). Extracts were frozen, freeze-dried and stored (-80°C). Metabolite extracts were derivatised with methoxyamine-HCl and MSTFA. For the analysis, a Shimadzu QP2010 Ultra GC-MS was used and for data analysis, AnalyzerPro The Unscrambler X and SPSS were used. Compounds were matched to an in-house library of metabolites and the NIST mass spectral database. Preliminary analysis by PCA distinguished metabolite profiles from LPK and Lewis rats for kidney (PC-1 79%) and liver (PC-1 42%) organ tissue. In the liver, approximately two-fold significant increases in four amino acids ($P \leq 0.016$) and four or more fatty acids ($P \leq 0.030$) in the LPK rat were determined. In kidney tissue, several amino acids were decreased in the LPK rat with fold changes of approximately 0.1-0.5 ($P < 0.001$). One amino acid increased (L-tyrosine; ≥ 6.7 fold change; $P < 0.001$), and several amino acids (hippuric acid, 1-methylhistidine, L-tryptophan) were present in the LPK kidney tissue but were not detected in the Lewis control samples. Four carboxylic acids were significantly decreased (0.1-0.7 fold changes; $P \leq 0.036$) in the LPK kidney samples including two (succinic and itaconic acids) which were not detected at all. Two carboxylic acids (malic and citric acids) were significantly increased (≥ 1.6 fold change; $P \leq 0.024$) in the LPK kidney tissue samples. Several fatty acids were significantly decreased (≤ 0.7 fold change; $P \leq 0.014$) in the LPK kidney compared to the Lewis control. Significant increases in monosaccharides were found for the LPK kidney tissue including several which were not detected in the Lewis samples. Urea was significantly increased in the LPK liver (~1.5 fold change; $P \leq 0.012$) and kidney (≥ 2.3 fold change; $P \leq 0.007$) tissue samples. Biochemical pathway analysis is underway to determine the significance of these results. In addition, these data are accompanied by urine and plasma samples which were collected weekly over a period of 12 weeks to determine significant changes in the disease progression. The longitudinal urine and plasma data analysis is underway and will provide an integrated data set for the study of disease progression in PKD. Untargeted metabolomic analysis reveals distinct metabolite profiles for PKD kidney and liver tissue with potential medical diagnostic implications.

POSTER 429

Metabolic profiling of tissue and biofluids – applications in the study of disease

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Metabolomics is an emerging field that offers huge potential for disease diagnosis, the evaluation of drug treatments and for understanding the pathogenesis of disease. By using mass spectrometry-based methods hundreds of metabolites can be detected in a given biological sample, providing a snapshot of metabolic activity at a specific time. The development of robust, reproducible methods for the analysis of multiple sample types is essential, to obtain a comprehensive picture of the metabolome. Determining the origin of the metabolites and disturbed biochemical pathways may provide important information regarding the pathogenesis of disease and the discovery of potential targets for treatment. In this study, we employed gas chromatography mass spectrometry (GCMS)-based methods to examine the metabolic profiles of multiple sample types including five different types of mouse tissue (liver, kidney, muscle, pancreas and gut). Multivariate data analyses were performed to determine differences between the metabolic patterns of these tissue types. The relationship between the organs and plasma were also investigated using hierarchical modelling. A novel GCMS-based method was used to perform metabolic profiling of liver, kidney, pancreas, muscle and gut. Only 10 milligrams of tissue was required for metabolite detection. Principal components analysis showed a clear separation between the metabolic profiles of the five tissue groups, showing that each organ has a unique metabolic profile. The contribution of different organs to the metabolic profile of plasma was also assessed. The analytical methods developed in this study, comparisons between the metabolic profiles of different sample types and the use of these methods in the investigation of disease processes will be discussed. The combination of tissue and biofluid metabolomics may prove a powerful tool in the discovery of potential targets for treatment and monitoring of disease. The first study to compare the metabolic profiles of five different tissue types and plasma using GCMS.

POSTER 430

Screening of the maternal serum metabolome for biomarkers of spontaneous preterm birth

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Preterm birth (PTB) is a major pregnancy complication which is responsible for significant perinatal morbidity and mortality. PTB can either be spontaneous or medically induced for maternal and/or fetal conditions. The development of spontaneous preterm birth (sPTB) is thought to be multifactorial, and it has proved difficult to identify women at increased risk. In the current study, we determined metabolite profiles using gas chromatography-mass spectrometry (GC-MS) in a nested case-control study, in order to identify early gestation biomarkers of PTB in maternal serum samples. Our aim was not only to identify biomarkers related to this reproductive outcome, but also to explore the underlying mechanisms of PTB. We designed a nested case (PTB 37 weeks of gestation, n=112). We utilised maternal serum samples from the Irish cohort of the Screening for Pregnancy Endpoints (SCOPE) biobank; these were obtained at 15 and 20 weeks' of gestation. Mothers who delivered preterm were matched to 2 sets of controls: one set was matched for maternal age only and the other for both maternal age and BMI. Statistical analysis of the clinical data was performed using Metaboanalyst. Samples were stored at -80 °C until analysis followed by extraction using 50% and 80% methanol. After extraction, the samples were subjected to methylchloroformate (MCF) derivatization and then analysed using GC-MS. There were no significant differences (PTB vs control, $P>0.05$) in metabolite profiles observed at 15 weeks of gestation. However, preliminary analyses identified significant differences in the metabolite profile obtained from maternal serum taken at 20 weeks of gestation. In 20 weeks' samples, 15 metabolites were significantly increased in PTB cases when compared with controls matched only by age ($P<0.05$). Nine metabolites were identified as significantly increased when PTB cases were compared with controls matched by age and

BMI. The discriminatory metabolites were organic acids, amino acids and antioxidants (P<0.05); seven were common for both sets of controls. The false discovery rate for discriminatory metabolites ranged from 0.18 to 0.25. 53% of PTB cases exhibited preterm premature rupture of membranes (PPROM) prior to sPTB. No discriminatory metabolites PPROM cases (vs. controls or vs PTB without PPROM) were identified. The research findings obtained from this discovery phase will be validated on a separate cohort using the same experimental design (the New Zealand based SCOPE biobank). Developing a high-throughput diagnostic tool has the potential to significantly impact both the diagnosis and subsequent treatment of PTB.

POSTER 431

Global Metabolomics of Complications in Pregnancy

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In 2011, the fourth leading cause of infant mortality was maternal complications during pregnancy. One increasingly common complication is gestational diabetes. Babies born to mothers with gestational diabetes have an increased risk of developing diabetes, obesity, or metabolic syndrome. Previous work has shown that a maternal increase in cortisol leads to an increase both in maternal glucose and fetal cortisol and glucose levels, and increases the incidence of stillbirth in the peripartum period. In this study, I used global metabolomics to investigate the role of cortisol in a sheep model of pregnancy. I hypothesize that increases in maternal cortisol lead to an increase in fetal cortisol and glucose, disrupting normal metabolic pathways of the maturing fetal heart. In a model of high stress pregnancy, 104 sheep serum samples were collected from 4 control, saline-infused ewes and 2 cortisol-infused ewes and their fetuses on gestational days 125, 130, 135, 140, and 142 to birth. 52 heart samples were collected following birth or death from the left ventricle, right ventricle, and/or septum in 4 control and 4 cortisol fetuses. 1H-NMR 1D and 2D data sets were analyzed using multivariate statistics and Statistical Total Correlation Spectroscopy (STOCSY) was used to identify potential biomarkers or pathways that are disrupted following stress. Metabolites were identified using COLMAR and confirmed using “spiking” methods in NMR. 1H-NMR spectroscopy of fetal heart reveals distinct metabolic differences between fetuses that underwent birth versus fetuses that did not, regardless of the treatment group. However, in the newborn lambs, phosphoethanolamine was significantly increased in hearts of control lambs relative to lambs from cortisol-treated ewes ($p<0.05$). This result suggests that increases in maternal cortisol lead to a disruption of lipid metabolism in the fetal heart, which may contribute to fetal mortality at term and that this disruption is only seen after delivery. Further work with a larger number of animals is needed for these results to be confirmed. 1H-NMR spectroscopy of maternal and fetal serum following cortisol infusion will be used to identify differences in TCA cycle intermediates, carbohydrate, and lipid metabolites. These results will be combined with fetal heart data, to gain a global perspective of metabolic changes that occur during complications in pregnancy in both the mother and fetus. Metabolomic findings will be integrated with phenotypic and genomic data, to begin to create a systems biology perspective of pregnancy, which will ultimately be translated to human umbilical cord blood with the ultimate goal of allowing the continued growth of the fetus in utero until full-term and development. Metabolomics is utilized to identify metabolic changes that occur in normal pregnancy, and how these changes are disrupted by complications.

POSTER 432

Potential metabolic biomarkers for chronic graft-versus-host disease

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Graft-versus-host (GVHD) is a multisystem disorder that is the major complication of allogeneic hematopoietic stem cell transplantation (HSCT). GVHD is an autoimmune-like disease where T-cells from donor recognize, are activated and attack tissues from host, causing severe morbidity and high rate of mortality. Whereas biomarker panels have been developed for the acute form of the disease, there is a lack of information about the chronic form (cGVHD). Chronic GVHD occurs in 40-80% of recipients and the diagnosis is essentially clinical after tissue damage. This pilot study intends to point potential blood biomarkers that could predict the onset of cGVHD before severe tissue damage. Blood serum samples from patients undergone HSCT were prospectively collected in four different periods: on the day of hospitalization (D-7), on the day of transplantation (D=0), during the neutropenic period (D+10) and D+100 post-HSCT. Samples were filtered at 3kDa filters and the serum metabolome from 18 patients was exploited by nuclear magnetic resonance. The acquisition of spectra was performed on an Agilent/Varian INOVA600 spectrometer operating at 1H Larmor frequency of 599.887 MHz and temperature of 298 K. Univariate and multivariate statistical analyses of metabolites profiles were performed by MetaboAnalyst 3.0 and Prism 6.0e. We identified and quantified 63 metabolites and performed the metabolomic profiling on days -7 (D-7), D=0, D+10, D+100, relative to transplantation day. No patient was diagnosed with cGVHD at blood collection time. Afterwards, from 18 patients analyzed, 6 developed cGVHD and 12 did not (cGVHD-free). Four metabolites were important to cluster cGVHD-free versus cGVHD groups, with predictive value for cGVHD: cystine, leucine (D+10 and D+100), phenylalanine and hypoxanthine (D+10). Cystine was more associated to cGVHD development than other metabolites ($p = 0.04$ two-way Anova, $p=0.004$ and $p=0.03$ t-test for day +10 and +100, respectively). Cystine, more than a biomarker, may be involved in the etiology of cGVHD. Cystine, essential for T cells activation and proliferation via dendritic cells, had its concentration increased in cGVHD group. Our results demonstrate the importance of biomarkers panels for predicting and understanding cGVHD, with the feasibility of prediction in early stages of disease, even where clinical symptoms are not yet evident, allowing therapeutic interventions even before tissue damage. Our results suggest that cystine could be the first biomarker that could predict cGVHD onset.

POSTER 433

Assessing injury responses in serum using a shotgun LC-MSE lipidomics approach

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High throughput yet comprehensive data-independent (MSE) lipidomics is useful in quickly assigning lipids to their classes, extracting structural information through the use of elution profiles of the precursor masses and the fragmentation profiles obtained in the high collision energy scan, and quantifying ions relative to the proper exogenous standards. The recent adaptation of this approach in LC-MS (liquid chromatography-mass spectrometry)-based lipidomics allowed us to quickly and efficiently assess injury after injection of Cesium-137 (137Cs) and Strontium-90 (90Sr) on the serum lipidome in mice. 137Cs and 90Sr are the 2 most feared internal emitters released into the environment after a nuclear or radiological disaster such as Chernobyl and Fukushima Daiichi. Lipids were extracted in chilled chloroform/methanol mixture containing non-endogenous lipid standards from mice sera collected at days 2, 3, 5, 20, and 30 post exposure to 137Cs or 90Sr (under federal and state guidelines). 2 µL injections on CSH C18 columns with UPLC solvents A (50% acetonitrile with 0.1% formic acid and 10 mM ammonium formate), and B (isopropanol/acetonitrile (90:10 v:v) with 10mM ammonium formate) were processed on a Xevo-G2-S QTOF (Waters Corp, Milford, MA) in ESI+ and ESI− modes over m/z of 50-1200 Da in MS and MSE channels. Data were processed in MassLynx and MetaboLynx, statistically

analyzed and annotated in MetaboLyzer, and compared against MS/MS databases for structural validation. We assessed changes in the sera of mice exposed to 137Cs or 90Sr over the course of 30 days via UPLC-QToF-MSE in order to assemble an exposure specific biomarker panel, which may help in the assessment of exposure after a nuclear/radiological disaster. More than 2500 spectral features were detected in both ESI modes combined in each exposure group. These ions were used to determine the changes in the serum lipidome of mice after exposure at different time-points/doses. Although there were several common ions which showed perturbations in their serum abundance in both cases, there were distinct differences. For example both treatments showed statistically significant changes in the serum levels of different classes of lipids, particularly choline-based phospholipids. In the case of 137Cs these changes reverted back to pre-exposure levels while with 90Sr there was no such adaptation or repair in the presence of continued irradiation. Statistical analysis suggested that in both exposure cases there was a global; in the case of 90Sr persistent; decrease in the serum levels of several phosphatidylcholines (PCs); PC(32:2), PC(32:1), PC(34:2), PC(34:1), PC(36:2), PC(36:1), and PC(36:0); and their derivative lysophosphatidylcholines (LPCs); LPC(16:0), LPC(18:2), LPC(18:1), and LPC(18:0). The most striking difference between the serum lipidomic signature of 90Sr and 137Cs was in the levels of triacylglycerides (TGs). While there was no significant changes with respect to serum levels of TGs post-137Cs-exposure, exposure to 90Sr induced statistically significant increases in the levels of six TGs; TG(50:12), TG(54:6), TG(56:7), TG(52:4), TG(56:5), and TG(61:6); as early as 2 days post-90Sr-injection. This may be due a decrease in lipoprotein lipase activity and lower uptake of TGs. In addition, the post-exposure serum levels of plamitic acid and linoleic acid showed a slight decrease with levels returning to pre-exposure levels by the end of the experiment in both exposure cases. Overall changes in the mice serum lipidome post 90Sr/137Cs exposure were studied for the first time, using an LC/MSE approach.

POSTER 435

What can we learn from a drop of blood from the Fanconi Anemia patient? An untargeted metabolomics approach

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Fanconi anemia (FA) is a recessive blood disorder characterized clinically by genomic instability, diverse congenital abnormities, bone marrow failure and cancer predisposition. Treatment with androgens and hematopoietic (blood cell) growth factors can rescue bone marrow failure temporarily, but curative treatment requires a bone marrow transplant. Prevention is key, but prospective disease markers indicating the development of blood cancer originating from the genetic defect in DNA repair genes are yet to be identified. A clear and comprehensive understanding of Fanconi anemia, a cancer prone disease, at a system-wide level and data extrapolation to the multitude of pediatric cancers is crucial for prognosis and the development of effective and targeted therapies. Mass spectrometry-based untargeted metabolomics/lipidomics was performed on a Xevo G2-S Q-TOF mass spectrometer interfaced with the ACQUITY UPLC system (Waters, Milford, MA, USA). Plasma (10 µL) from patients with FA (n=7) and age/gender matched controls (n=7) were extracted with various solvent systems to obtain a complete metabolome and subjected to our well-developed ultra-high performance liquid chromatography-mass spectrometry (UPLC-MS) platform. Univariate and multivariate analyses were performed in the selection of biomarkers from resulting metabolomics data. Key metabolites were identified by database searching and confirmed with authentic standards. Furthermore, quantitative assays for these biomarkers were developed to provide accurate concentrations. Putative biomarkers were assessed by receiver operator characteristic (ROC) curve analysis for their performance in prognosis of FA and cancer development. Our preliminary MS-based global metabolomics study from FA plasma revealed the existence of several metabolite candidates that together comprise the FA signature. Principal component analysis, a multivariate statistical technique that simultaneously analyzing the interrelationship among thousands of metabolites to cluster data thus defining group differences, clearly shows that several lipids in the blood of

FA patients are uniquely different from those of health controls. Markers elevated in FA patient blood include potent, bioactive lipid species which were previously reported to be associated with a number of cancers. Quantitative analysis of these lipid species were employed to validate results from untargeted comparative analysis. Based on area under ROC curve (AUC), a few sensitive and specific markers were present in blood, which could be potentially used in clinical application. An untargeted metabolomics/lipidomics study of FA patient plasma yielded potential biomarkers to facilitate FA diagnosis and therapy monitoring.

POSTER 436

The use of spectral trees as a novel approach in identification of adenosine deaminase deficiency metabolites in human urine

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Adenosine deaminase deficiency (ADA) belongs to the various group of inborn errors of purine metabolism. These diseases express large variety of neurological, immunological, heamatological and renal manifestations. Accumulation of toxic intermediates or depletion of nucleotides deepen the disorders' pathogenesis. Most of the defects are inherited in an autosomal recessive manner (Balasubramaniam et al., 2014). Based on our preliminary studies on capillary electrophoresis with UV detection, many purine metabolites were found. Nevertheless, no sufficient identification tool was available. This was overcome by novel approach of spectral trees, which uses multistage fragmentation to identify different metabolic modifications. Spectral tree is an intuitively organized multistage tandem mass spectra acquired at different collision energies, and techniques (www.mzcloud.org). This approach allows us to see the unmodified structure of the metabolite at lower MSn level and by "bottom-up" strategy get the individual metabolic modifications – dentifying the molecule. Data were acquired using untargeted metabolomics approach by UHPLC coupled with high resolution tandem mass spectrometer Orbitrap Elite. Data were evaluated by recently introduced software Compound Discoverer and statistically processed using R-software. Number of potential metabolites of adenosine, inosine and deoxyadenosine found by Compound Discoverer was 36. Most frequent metabolic transformations were methylation, oxidative deamination and desaturation. All metabolites were acquired with mass error less than 0.5 ppm. For comparison four ADA patient samples and twenty healthy volunteers were used. By using the approach of spectral trees metabolites were successfully confirmed. In the future this method can represent significant tool for identification of metabolites with potential diagnostics importance. This project was supported by NPU I (LO1304), Czech Science Foundation Grant I 1910-N26 and CZ.1.07/2.3.00/30.0004 Grant. Utilisation of Spectral tree strategy for identification of new biomarkers of metabolic disorders.

POSTER 437

The global lipidomic profiling of G6PD-deficient embryos

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Glucose 6-phosphate dehydrogenase (G6PD) is the rate-limiting enzyme in pentose phosphate pathway,

which is a major source of NADPH for reductive biosynthesis, including lipid synthesis. Human G6PD deficiency, known as favism, is manifested by hemolytic anemia upon exposure to oxidants and anti-malaria drugs. Contrast to the well-known knowledge in red cell physiology, the essential role of G6PD in embryonic development is an uncharted territory. Recently, we have established a G6PD-deficient C. elegans model and shown that G6PD affects embryogenesis. In this study, we asked two major questions: Firstly, what are the characteristics of G6PD deficiency in embryonic development in C. elegans? Secondly, whether lipid metabolism plays a role in G6PD deficiency-induced embryonic defects? C. elegans fed on E. coli bearing plasmid of mock and RNAi against G6PD or lipid metabolic genes were harvested at adult stage. The gravid adult worms were bleached with alkaline and hypochlorite to isolate embryos. These embryos were homogenized by sonication and subjected to lipid extraction according to Folch's method. The lipid fraction was then separated by ACQUITY UPLC system (Waters) using CSH C18 column (Waters) and coupled with a mass spectrometer (SYNAPT HDMS G1, Waters). After data collection, the data was analyzed by MassLynx (Waters) and MetaboAnalyst (U. Alberta). Potential identification of C. elegans lipid candidates was initially searched by using Human Metabolome Database (U. Alberta). Embryos isolated from G6PD-deficient C. elegans parents demonstrated significantly reduced G6PD activity compared with mock embryos. Under Differential Interference Contrast (DIC) microscope, these embryos displayed distinctive morphological alterations in response to osmotic stress. They also exhibited morphologic compromised egg shell structure and disrupted permeability barrier. Increased permeability to several small molecule fluorescent dyes was also observed. Furthermore, there were several embryonic defects associated with altered membrane activity, such as failure of forming cortical ruffling and pseudo cleavage, absence of perivitelline space, and symmetric division. More importantly, these embryos took longer to grow and arrested before hatching. After LC-MS data collection and processing, the principle component analysis (PCA) plot showed that G6PD-deficient embryos were well separated from mock embryo. To investigate the relationship between G6PD and lipid metabolism, representative lipid metabolism mutants, including fat-1, fat-5, and fasn-1(RNAi) were also included in the analysis. The data indicated that the lipid profiling of G6PD deficiency was closer to that of fasn-1(RNAi) mutant and away than that from fat-1 and fat-5 mutants in PCA plot. This result was consistent with the observation that G6PD-deficient and fasn-1(RNAi) mutant embryos displayed enhanced permeability in contrast to embryos of fat-1 and fat-5 mutants. Data analysis of mock and G6PD deficiency by MassLynx and MetaboAnalyst in both ESI-positive and negative modes showed that G6PD deficiency significantly up-regulated LysoPC/LysoPE and down-regulated PC/PE, suggesting the altered membrane lipid composition may contribute to embryonic defects caused by G6PD deficiency. The lipidomic profiling of nematode embryos provides useful insights into the relationship between G6PD and lipid metabolism in embryonic development.

POSTER 438

Metabolic phenotyping the renal transplant surgical journey

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Successful renal transplantation not only improves patients' quality and duration of life, but also confers a substantial economic healthcare cost saving. With the growing burden of end-stage renal disease and the requirement for renal replacement therapy, strategies to augment transplant success and subsequent graft survival become more vital than ever. Unfortunately, conventional clinical measures are limited specifically in terms of post-operative care/monitoring, during the initial days following transplantation, where susceptibility to complications is high and early corrective intervention lifesaving. Growing in prominence however, Systems Medicine represents one such powerful strategy, and in particular the field of metabolomics. A unique study has been establish to metabolically phenotype prior to (24 h) and post

(days 1–5) surgery living donor renal transplantations, performed at the Imperial College NHS Trust Renal & Transplant Centre (London, UK). Using an advanced multi-platform analytical strategy (i.e., combined 1D & 2D Nuclear Magnetic Resonance (NMR) spectroscopy, untargeted lipidomic and targeted oxylipin Mass Spectrometry (MS) and chemometrics), donor and recipient (n = 100 pairs) urine and plasma metabolic profiles were obtained, and subsequently integrated and modelled, with the ultimate aim to devise an objective means of characterising renal function post-transplantation and to stratify patients on the basis of likelihood of complications (such as delayed graft function, rejection episodes or disease recurrence). The focus of this presentation will be directed towards the real-life, initial application of an exhaustive metabolomic approach in clinic and surgery, exemplified through key cases from initial untargeted exploration (hypothesis generating) to targeted analysis (hypothesis testing), for both healthy donors and ill recipients. The implementation of advanced chemical techniques (NMR and MS) for urine and plasma metabolite profiling will be described, along with the subsequent multivariate approaches necessary to successfully interpret and correlate markers or patterns that define particular class information. Both unsupervised, such as principal components analysis (PCA), and supervised, such as partial least squares (including potential orthogonal signal correction) regression and discriminant analysis ([O-]PLS[-DA]), chemometric techniques will be demonstrated as a means to stratify sub-populations attributed to numerous causes, both endogenous and exogenous, for example, from underlying physiology, to clinical comorbidities and even therapeutic drug administration. Finally, multimodal data integration with conventional clinical parameters will briefly be discussed. Specific examples include: Characterisation of common sources of variance (i.e., surgery, time and pre-transplant status) using univariate and multivariate discriminant analysis on both by 20 quantified core metabolites from 1D 1H NMR profiling of urine (donors and recipients) as well as 48 quantified oxylipins from SPE-UPLC-MS/MS profiling of plasma (donors and recipients). Univariate and multivariate regression between high-resolution 1D 1H NMR of plasma (donor and recipients) and current clinical measures (i.e., haematology, coagulation and biochemistry), in addition to multi-block O2-PLS integration between datasets, to improve interpretation and understanding. Statistical exploration of conventional clinical variables with regards to classification of complications, as a comparative baseline for this metabolomic approach. First comprehensive display of a multi-platform strategy to metabolically phenotype the renal transplant surgical journey for donors and recipients.

POSTER 439

Biomarker Study In A Urea Cycle Disorders Mouse Model

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Ammonia derived from protein catabolism is highly neurotoxic and is removed by conversion to glutamine for transport to the liver. In hepatocytes, ammonia from amino acids enters the urea cycle and is converted to urea, which is both non-toxic and excretable. Urea cycle disorders (UCDs) are inborn errors of metabolism where deficiencies in the urea cycle enzymes inhibit this conversion. UCD patients suffer periodic hyperammonemic events leading to permanent CNS damage, neurocognitive impairment, and even death. Discovery of new therapies for these diseases can be greatly accelerated by relying on phenotype-linked metabolic biomarkers for establishment of screening assays and relevant animal models. Identification of biomarkers is based on global profiling carried out using chromatographic separation coupled with high resolution accurate mass spectrometric detection (HRAM). Data processing is done using sophisticated peak picking software, statistical packages, and in-house software packages. Through the use of global metabolomic profiling and statistical analysis we are able to identify relevant biomarkers of both the disease and treatment of UCDs. Further examination of the sizable list of potential biomarkers with trend analysis allows for a focused subset of biomarkers that follow disease status and subsequent treatment. These biomarkers represent not only urea cycle metabolites but also metabolites not typically associated with the urea cycle. Comparison of biomarkers across brain and plasma samples combined with pathway analysis allows for a greater understanding of the biology involved with UCDs. Following disease progression and treatment across multiple sample types with trend analysis.

POSTER 440

Profiling acetaminophen dose-dependent metabolites in rat plasma by an untargeted UHPLC-HRMS/MS approach

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Acetaminophen is the main cause for acute liver failure in North America. This is due to its reactive metabolite-related hepatotoxicity. In order to better characterize the metabolic perturbations occuring with acetaminophen dosing, an untargeted metabolomics approach was employed for analysing rat plasma samples. Using two complementary chromatographic separations coupled to high resolution tandem mass spectrometry, we were able to access thousands of features in rat plasma for probing changes to the metabolome as a function of the dosing level of acetaminophen. Four dosing levels of acetaminophen (n=3 each) were administered to rat and 24h post-dose plasma samples were analysed by LC-MS/MS. Samples were prepared by methanol precipitation and injected onto an ultra-high performance liquid chromatography system coupled to a quadrupole-time of flight mass spectrometer (Nexera UHPLC-AB Sciex 5600 TripleTOFTM system). Extracts were separated on PFP (Phenomenex) and T3HSS (Waters) columns for comparison of results in positive and negative electrospray modes. Acetaminophen-dosed rat samples were compared to pre-dose samples as controls. Statistical analysis was performed and features having unique patterns based on acetaminophen dose were extracted for detailed analysis. In a follow up study, time-course samples were analysed for the metabolites of interest, to better understand pharmacokinetic profiiles associated to these perturbations. PFP and T3HSS columns were found to give complementary metabolomic separations. Also, some metabolites were found uniquely in one ionization mode and others were detected in both modes. Following statistical analysis, many features were found to have interesting profiles, including increasing levels with dose (and not present in control samples) as well as decreasing levels with dosing level (and present in control samples). Metabolite identification was achieved by accurate mass and MS/MS matching with metabolite databases (METLIN and HMDB) as well as retention time matching, when standards were available. Interestingly, many features of interest represented novel metabolites not previously found in metabolomics databases, however, by studying MS/MS patterns, tentative structures for these compounds were elucidated. Elucidating dose-dependent metabolomic signatures related to acetaminophen dosing for understanding perturbations involved in hepatotoxicity

POSTER 441

Large-scale metabolomic analyses revealed novel biomarkers of ischemia/reperfusion injury and potential mechanisms of remote ischemic preconditioning in kidney.

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Ischemia and reperfusion (IR) injury is a multifactorial process detrimental to kidney graft function. Therefore, understandings of the mechanisms involved in IR injury are essential for therapeutic strategies to improve the outcome of kidney transplantation. Remote ischemic preconditioning (RIPC) is a strategy inducing resistance in the target organ against the oxidative stress and injury caused by IR. RIPC harnesses the body's endogenous protective capabilities through brief episodes of IR applied in remote organs from the target. Many researchers found protective effects of RIPC in heart, however, there has been few study in kidney. Although there are some studies regarding renoprotective action of RIPC in vivo, the window of protection representing RIPC efficacy and their mechanisms have not been fully elucidated. Total 30 mice (male, 9 wks) were subjected to this study, and randomly divided into the sham,

IR, Late RIPC + IR (RIPC, 24 hr before IR), or Early RIPC + IR (RIPC, 2 hr before IR) group. We confirmed histological spectrum of IR injury and its amelioration by RIPC in kidney. Then we performed large-scale metabolome analyses through the Agilent LC/Q-ToF MS system to identify significantly changed metabolites after renal IR injury as compared to controls, and those showing protective effects of RIPC from the IR injury. Collected kidney, serum, and urine samples were taken before and after treatment followed by large-scale metabolomics combined with multivariate data analyses and pathway analysis. The IR definitely showed tubular injury, and both late RIPC + IR, and early RIPC + IR groups represented ameliorated injury after the insult (H&E staining). Also, renal IR injury produced changes of the metabolome in kidney, serum, and urine specimens: 13, 125, and 9, respectively. Furthermore, several metabolites that reflect the effect of RIPC were identified (7 in kidney, 10 in serum, and 3 in urine). Taken altogether, IR induces osmotic stress, oxidative stress, vasodilatory effects, membrane disruption, and immune reaction, and RIPC effectively reduces renal damage after IR. More importantly, potential mechanisms of both time window of protection were different. We elucidated that metabolites indicating renoprotective effects were different in two RIPC group: reducing of inflammation signaling, and restoring cellular energy metabolism. These results indicate that late RIPC and early RIPC are related with different mechanisms concerning IR injury attenuation. These results may well be applied to therapeutics against oxidative stress injury in human through non- or minimally invasive diagnosis. The first study revealed novel biomarkers of renal IR injury and its different mechanisms of RIPC according to time window.

POSTER 444

Lipidomics of nonalcoholic fatty liver disease and nonalcoholic steatohepatitis in a mouse model via LC-HRMS

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Approximately one-third of the US population is affected by nonalcoholic fatty liver disease (NAFLD), with half advancing to nonalcoholic steatohepatitis (NASH). The lipotoxic environment associated with obesity is thought to contribute to hepatic insulin resistance and NAFLD. However, the impact of 'lipotoxicity' on hepatic mitochondrial metabolism and the transition of NAFLD to NASH is not clear. A disproportionate increase in toxic lipid byproducts along with sustained increases in TCA cycle flux suggests incomplete fat oxidation in mouse NASH liver. This research investigates changes in hepatic lipid composition in a diet-induced mouse model transitioning from NAFLD to NASH. Defining biomarkers in the liver may indicate potential biomarkers in the blood, which would allow less invasive analysis, earlier diagnosis and earlier treatment. Mice (C57/BL6) were fed either a control diet or a high fructose, trans-fat diet (TFD) for 8 weeks to induce NAFLD, or 24 weeks to induce NASH. Severity of hepatitis was substantiated by liver histology. In vivo fasting hepatic fluxes were measured by stable isotope NMR and isotopomer analysis. The lipids were extracted from liver tissue using the Folch method and analyzed by LC-MS based targeted and untargeted metabolomics. Samples were analyzed via UHPLC-HRMS with reverse phase chromatography. Data were acquired full scan and all ion fragmentation (AIF) with positive and negative ion mode switching. Data were processed and visualized using XCMS in R. Compounds were normalized to internal standards within the same lipid class and protein content. Previously collected data corroborate with literature demonstrating the increased prevalence of diacylglycerols (DG) and ceramides (Cer) in NAFLD and NASH. Ceramides increase with NAFLD but not further in NASH, whereas DGs continue increasing with disease severity. Total Cer content increased from 8 wk control mice (1.7±0.1 µmol/gram liver protein) compared to 8 wk TFD mice (3.2±0.1). Additionally, total Cer content increased from 24 wk control mice (1.8±0.1) to 24 wk TFD mice (3.4 ± 0.2). Total DG content increased from 3.5±0.3 to 9.7±1.5 µmol/gram liver protein in 8 wk control mice and 8 wk TFD mice, respectively. Moreover, 3.1±0.3 µmol/gram liver protein in 24 wk control mice increased to 16.6±1.2 µmol/gram liver protein in 24 wk TFD mice. The DG fold changes between NAFLD and NASH are significant in 14 out of 20 DGs ($P < 0.05$). These data suggest the DG concentrations increase in both NAFLD development and further in NASH development. In 8 out of 9 ceramide species, the increase in signal among NAFLD mice was significant,

whereas 6 out of 9 ceramide species had significant increases among NASH mice. Despite these individual increases, the fold change between the NASH and NAFLD was significant only in 3 of 9 species (HexCer16, Cer22, and Cer20). This suggests that the ceramide levels increase mostly during NAFLD development, but not significantly more in NASH development. Untargeted analyses were performed to expand metabolomics and identify other compound classes and signatures that contribute to the metabolic transition from NAFLD to NASH. Principal components analysis has shown separation of healthy and diseased samples with progressive separation as the disease increases in severity. This and other multivariate analyses are being used to identify individual species as biomarkers that indicate disease transition; thus aiding in earlier diagnosis. The first use of LC/MS using this diet induced mouse model to study the transition from NAFLD to NASH.

POSTER 445

Phosphatidylethanolamine and Triacylglycerol play important roles in fat overload induced metabolic perturbation on HepG2 cells

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Non-alcoholic fatty liver disease (NAFLD) is characterized by abnormal fatty acid (FA) accumulation in liver without excessively consuming alcohol. In vitro exposure of liver cells to high concentrations of free FA results in fat overload which promotes inflammatory and lipotoxic responses. In this study, we aimed to demonstrate the fat overload-induced metabolic perturbation. The human HepG2 cells were treated with several free fatty acids, including the palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0) and oleic acid (18:1). Fat accumulation, lipotoxic effect, apoptosis, and production of inflammatory were measured. Metabolic profiling was performed using UPLC-Q-TOF/MS and data analyses were calculated by MassLynx, SIMCA-P and HMDB for processing, identifications and pathway analysis. Increasing concentration of FAs and treating time showed different lipotoxicity. There was more fat accumulation with monounsaturated FAs than saturated FAs treatment. Comparing with lipid metabolic changes of C16:0 and C16:1 treatment, diacylglycerol (DG), ceramide and free FA were significantly increased on C16:0 treatment. The concentrations of phosphatidylethanolamine (PE) and triacylglycerol (TG) were increased on C16:1 treatment. IL-8 RNA level as an inflammatory marker was only enhanced with increased C16:0 treated time. This phenomenon demonstrated that saturated FAs induced higher lipotoxicity caused by more accumulation of DGs without undergoing storage of TGs. There may be anti-inflammatory action on monounsaturated FAs treated HepG2 cells simulated NAFLD. Focus of this study elucidated PEs or some unique metabolites in PE generated pathway associated with anti-inflammatory action and anti-Lipotoxicity.

POSTER 446

Investigation on the effects of piceatannol on the high-fat-diet-induced hyperlipidaemia using rat models by LC-TOF-MS serum metabolomic profiling

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Cardiovascular Diseases (CVDs) are responsible for the number one cause of death globally. Piceatannol, a metabolite of resveratrol found in red wine, was demonstrated to have significant health benefits on antioxidant effects, inhibition of adipogenesis and improving endothelial function. However, there is a lack of comprehensive studies about the protective effect of piceatannol on CVD under the high-fat-diet (HFD) induced hyperlipidaemic condition. Therefore, a study was conducted to investigate the protective effects of piceatannol on rats fed with HFD. A metabolomics approach was conducted to further investigate the putative role of piceatannol in ameliorating atherogenesis in experimental animals (rats) by evaluating the serum profiling via ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass (LC-TOF-MS). Thirty-two 3-month-old male Sprague-Dawley rats were

randomly divided into 4 groups: normal diet (Control), high-cholesterol diet (HCD), high-cholesterol diet with simvastatin (SIM, 3 mg/kg bw per day), piceatannol (PCT; 10mg/kg bw per day) for 30 days. Serum samples were obtained at the end of 30-day treatment. Lipid profiles were measured by biochemical analyzer. Serum metabolites were analysed by LC-TOF-MS. QC samples were analyzed periodically to monitor the reproducibility and repeatability of the analytical method throughout the entire study. Centroided and integrated raw mass spectrometric data of serum were processed by MassLynx V4.1 and MarkerLynx. Partial least squared discriminant analysis and orthogonal partial least-squared discriminant analysis were used to identify the metabolites contributed most in separating the data from different groups. The atherogenic index [(total serum cholesterol – HDL-C) / HDL-C] is a parameter to measure the risk of coronary heart disease. The result shows that a significant elevation of atherogenic index was observed in the HCD group ($P < 0.001$) as compared to the control group. Administration of either simvastatin or piceatannol could significantly decrease the atherogenic indexes as compared to that of the control group (SIM: $P < 0.01$; PCT: $P < 0.01$). It suggests that piceatannol possesses atherosclero protective potential in the current experimental setting. The serum metabolite profiles show a clear distinction of control, HCD and PCT groups via orthogonal partial least-squared discriminant analysis. More than 20 metabolites responsible for the differences between groups have been identified and the results suggested that the therapetic effect of piceatannol may involve the regulation of glycerophospholipid metabolism and secondary bile acid biosynthesis. Piceatannol can lower the atherogenic index by regulaing of glycerophospholipid metabolism and secondary bile acid biosynthesis via metabolomics approach.

POSTER 448

Identification of serum diagnostic markers in polycystic ovary syndrome (PCOS) using nuclear magnetic resonance and liquid chromatography-mass spectrometry metabolomics approach

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Polycystic ovarian syndrome (PCOS) is the most common endocrine and metabolic disorder in women of reproductive age, with a variable prevalence ranging from 5-10%. Clinical manifestations include oligomenorrhea or amenorrhea, hirsutism and infertility. The disease is primarily considered as an outcome of physiological imbalance of the metabolic homeostasis and endocrine system that affects several body systems and can cause significant long-term health disorders. There is no universally accepted specific diagnostic test that can be recommended for diagnosis of PCOS. Extensive research is still ongoing to identify markers and understand better pathophysiology of the disease. This study focuses on the use of both, nuclear magnetic resonance (NMR) and liquid chromatography-mass spectrometry (LC-MS/MS) based metabolomics to identify valued diagnostic markers for PCOS. Serum samples were collected from 25 PCOS women (according to Rotterdam criteria 2006; ≥18yrs to ≤40 years) and from 25 women reporting for tubal ligation (controls). For NMR, samples were mixed with D2O containing reference and Carr-Purcell-Meiboom-Gill spin-echo spectra obtained, following which the data was processed and subjected to statistical analysis. Prior to LC-MS/MS analysis, metabolites were extracted using methanol extraction method. MS data were acquired using a QTRAP triple-quadrupole mass-spectrometer equipped with a HPLC system (HILIC column). Multiple reaction monitoring was used to acquire targeted MS data for specific metabolites in the positive ionization mode. Statistical analyses were performed and principal component analysis (PCA), partial least squares discriminant analysis

(PLS-DA) and orthogonal-PLS-DA were applied to discriminate between the groups. Statistical analyses were performed separately for both NMR and LC-MS/MS data. Several multivariate analyses, including PCA and supervised higher models, i.e. PLS-DA and OPLS-DA have shown highly distinct classification based on the differently expressed metabolites in serum from PCOS as compared to controls. High R2 (goodness of fit) and Q2 (predictive ability) values were achieved for both, NMR (PLS-DA; R2 0.93 and Q2 0.81), (OPLS-DA; R2 0.91 and Q2 0.83) and LC-MS/MS data (PLS-DA; R2 0.95 and Q2 0.7), (OPLS-DA; R2 0.95 and Q2 0.69). In the permutation test, R2 and Q2 values were significantly higher than the permutated models, indicating good predictive ability for both the metabolomics techniques. Several metabolites which contributed most towards this differentiation were identified. Fold change and variable of importance (VIP) score were used for this purpose. Finally, receiver operating characteristic (ROC) curve was used to screen the metabolites with highest accuracy in predicting PCOS. A total of eight amino acids and energy metabolites were found to have the maximum altered expression in PCOS as compared to controls. While NMR could identify 3-hydroxybutyric acid, lysine, lactic acid and histidine, LC-MS/MS detected L-arginine, L-citruline, xanthine, L-histidine as potential metabolites Next we propose to validate these markers in a randomly selected cohort of PCOS women. We also suggest that there is a need to use both the powerful metabolomics tools, NMR and LC-MS/MS concomitantly for the development of robust diagnostic markers. Systematic and exhaustive examination of metabolites using these two complementary techniques enhances the likelihood of identifying metabolites which are significantly dysregulated, reproducible, and possess sufficient sensitivity, specificity and accuracy in distinguishing disease from controls. Using complementary NMR and LC-MS/MS metabolomics tools, eight distinct metabolites are identified which can accurately identify PCOS patients

POSTER 449

Metabolic phenotyping discovers endogenous associations in under the limit salbutamol misuse in sports

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Current anti-doping analytical methods are tailored mainly to the identification of known drugs. This causes difficulties in rapidly reacting to emerging threats such as designer drugs, biological therapeutic agents and technologies. Biomarkers are considered as a promising approach for the fight against these threats to sport. The main purpose of this study was to find surrogate biomarkers induced by the intake of small amounts of the model compound salbutamol and explore a sensitive approach to help screen for possible drug misuse. Urine samples (91) from athletes with detectable salbutamol (30, which were still under the official reporting limit) and negative samples (61) were analysed using a UHPLC Q-Exactive Orbitrap MS in both positive and negative ionization modes. A third group was created by spiking salbutamol into 30 of the negative urine samples to exclude biological and analytical confounding. Data was then analysed in XCMS to get metabolic features. Orthogonal Partial Least Squares - Discriminant Analysis (OPLS-DA) was performed to select features correlated to detectable salbutamol ($p(corr) > 0.5$) and Receiver Operating Characteristic (ROC) curve was performed to measure the predictive potential of the markers. Univariate analysis including boxplot, Mann-Whitney U test and Spearman's correlation was conducted on selected markers. A total of 7,000 metabolic features were obtained by XCMS. OPLS-DA revealed a feature, identified as hypoxanthine, which was increased with salbutamol. The ROC curve of hypoxanthine returned an AUC of 0.79. Univariate analysis showed that hypoxanthine concentration was significantly greater ($p < 0.001$, Mann-Whitney U test) in the salbutamol detectable group and correlated to salbutamol ($r = 0.415$, $p < 0.01$, Spearman's correlation). These results showed hypoxanthine could be a potential surrogate marker of salbutamol misuse. Hypoxanthine is an end product of purine metabolism which reflects ATP consumption, indicating that salbutamol might affect purine metabolism in "under the limit" samples. This approach should be applied to a larger cohort of doping substances in controlled longitudinal experiments. Initial screening of urine samples from sports competitors to target misuse via surrogate markers.

POSTER 450

Development of Protocol for Global Metabolomics in Clinical and Cohort Study by LC-MS

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Metabolomics is an omics study to examine a whole set of small molecules in biological samples, and may be able to detect subtle changes in metabolic pathways and the deviation from the homeostasis before the manifestation of phenotypic changes. Thus, metabolomics can be a promising avenue to identify predictive or prognostic biomarkers. Although the quality of metabolomic analyses by mass spectrometry (MS), especially that of global metabolomics (G-Met), depends on instrumentation, potential bottlenecks still exist in several basic elements in a metabolomics workflow as follows: sample quality, method development, data normalization, and metabolite identification. Therefore, we decided to establish a precise protocol for G-Met of human plasma and urine to overcome the difficulties often associated with those samples. In our G-Met protocol, samples are deproteinized and filtered in a 96-well plate format by an automated liquid-handling system (HAMILTON, Starlet). The analytical systems for G-Met performed with an UPLC-Q-TOF/MS (Synapt G2Si, Waters) using a C18 (Acquity HSS T3; 150 mm x 2.1 mm i.d., 1.8 μm) column separation, and a LC-Q-FT/MS (QExactive, Thermo Fisher Scientific) using a HILIC (ZIC-pHILIC; 100 mm x 2.1 mm i.d., 5 μm) column. All data obtained from two systems were imported to the software (Progenesis QI, Nonlinear), respectively, for alignment the chromatograms and picking the features. The information of retention and m/z of features were imported to our original software. Normalization protocol was also developed to correct intra-batch and inter-batch difference. More than 6,000 features were detected totally in human plasma after alignment and peak picking using Progenesis QI with four analysis using C18 (pos and neg) and HILIC (pos and neg) condition. Then, about 2,000 metabolites are identified by database search. At first, we checked the stability of metabolites in blood sample. Typical types of phospholipids and phosphoinositol compounds from blood sample that was stored at room temperature were significantly increased ($p < 0.05$) with time dependency (0-48 hours). Second, we evaluated our normalization protocol using three plates (264 sample) G-Met analysis. The difference of inter-plate distribution on PCA analysis was improved after treatment of the normalization. Then, we applied the G-Met protocol on a small scale of clinical sample analysis (female pregnancy) for extracted candidates of biomarkers, and their relative quantities are evaluated by clustering analysis and multivariate analyses (i.e. PCA, PLS-DA, and OPLS-DA), and typical prostaglandins and steroids were decreased along with the period of pregnancy. Finally, we also applied on the cohort plasma analysis to develop the reference of metabolites. The frequency of samples in each features (retention time and m/z) were installed in our software. These protocols appeared to be useful for discovery and development of predictive and prognostic biomarkers for obstetric complications.

POSTER 451

When 5,674 samples are just a pilot study….

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The profile of circulating lipids reflects the body's energy homeostasis in response to nutrition, metabolic health and lifestyle. The response is influenced by the genetic make-up of an individual leading to distinct interpersonal differences in lipid metabolism and the ability to handle for instance the consumption of excess energy which can lead to the development of cardiometabolic diseases. We have therefore established an open-profiling method for lipids that is robust, reproducible, and appropriate for large-scale epidemiological sample-sets to allow the study of the association between genetic variances with lipid metabolism. We applied this lipid-profiling method to 5,674 samples from the Pakistan Risk of Myocardial Infarction Study (PROMIS) to investigate how metabolome/genome interactions influences the risk of developing cardiovascular disease. Samples were ran in balanced randomisation across the study ensuring that each batch of 80 samples was representative of the whole study. Serum samples were extracted using an automated 8-head liquid handler, working with a 96-well format and resulting in an organic phase upper layer. This organic phase was diluted with a mixture of isopropanol/methanol with 7.5 mM NH4Ac solution. Lipid profiling was performed by chip-based direct infusion (triversa Nanomate) coupled to benchtop Oribitraps to obtain high resolution mass spectra in both positive and negative mode. The resulting spectra were converted to mzXML and processed using an in-house algorithm based on XMCS that uses a list of 1400 theoretical m/z of possible lipids. Here we present the data processing method and results from the quality control to demonstrate the feasibility of open profiling of lipids in such large studies. A quality control sample (QC) was created by pooling 100 µl of 200 samples. A sub-set of the pooled sample was diluted with phosphate buffered saline (PBS) solution to two different concentrations giving three different QCs (QC1 was undiluted, QC2 was 1:1 diluted, and QC3 was 1:3 diluted). Each plate had 4 replicates of each QC level and 4 blanks, leaving room for 80 samples. The different QC levels where then used to determine which signals were biologically relevant. The First step in the data processing was the removal of signals with poor signal strength (lower than in the blank) and signals that were in less than 90% of QC samples detected across the entire study. Then signals with a poor Pearson correlation coefficient in the QC samples ($r < 0.95$) were removed. For each sample the signals were normalised against the total signal strength. The last step was the removal of any signals with a CV of more than 25%, resulting in a list of 207 lipids with unique mass-charge ratios and identifiers (average CV 13.44%, median 11.61%). The few lipids that were retained and are above the 25% cut-off line correspond to internal standards. This demonstrated that normalisation gave reproducible data on a par with other high throughput metabolic profiling methods. The profiling was possible within a 15 week period. As these results of this pilot study on n=5,674 are on par with smaller studies in our lab (n=1,500 and n=2,300 samples) we are confident that this approach can be applied to very large scale studies and we will discuss plans to expand this analysis to a study of 50000 individuals. A high throughput method for very large scale epidemiological studies that was applied to a study of 5764 samples.

POSTER 452

High-Throughput Metabolic Profiling in the Epidemiological Study of Metabolic Disease

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Type 2 diabetes (T2DM) and cardiovascular disease (CVD) are complex disorders where the genome interacts with the exposome to determine risk. From Genome Wide Association Studies (GWAS) of both

T2DM and CVD, in most individuals the genetic risk consists of a number of genes contributing a small amount to that risk, necessitating large scale cohort studies (>1000 samples). In contrast diet is a major contributor to the exposome and can be followed by metabolomics. In this study we describe a large scale metabolomic/genomic study of the gene-lifestyle interactions in the development of cardiometabolic diseases by combining information on metabolic pathways through the detailed profiling of lipids and metabolites with GWAS data, providing insight into the causes of these metabolic disorders. We have conducted a metabolomics investigation of a large epidemiological cohort (~11,500 individuals) where each individual is profiled using a targeted metabolomic approach using the Biocrates p180 kits followed by LC-MS/MS MRM based analysis. The instrumental method utilizes both a reverse phase, UPLC method for the absolute quantification of 42 metabolites, and a flow injection method to measure and relatively quantify 140 metabolites, which are primarily lipids. Currently, individuals are being analysed for associations with other factors being measured: genomic analysis using the Affymetrix Axiom BioBank chip providing information on 250,000 SNPS and a variety of lifestyle and clinical measurements (such as diet, physical activity, body fat distribution, liver function, insulin sensitivity and disease status). The scaling of both instrumental and data processing methods in this study requires the careful consideration of critical details important for high-throughput liquid chromatography and mass spectrometry (e.g. minimizing the need for instrument maintenance via curtain gas settings and maintaining an accessibility of raw, informative signal data important for quality of interpretation during complex data processing methods). This allowed an analysis of ~600 samples per week with the entire cohort analysed in 13 months. The average coefficient of variation across quality control samples of the Biocrates kit assay is ~ 17% for 42 of the absolute quantification metabolites and 95 of the relatively quantified metabolites. The large number of human samples investigated with a combination of mass spectrometry, genetic, and clinical/lifestyle measurements is novel.

POSTER 453

Metabolomic assessment and the use of a novel Na+-taurocholate cotransporting polypeptide inhibitor for the treatment of hepatitis B in humans

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Metabolomic monitoring of endogenous biomarkers is of increasing importance for the assessment of drug safety and efficacy during clinical drug development. Myrcludex B, a novel lipopetide-based entry inhibitor for the therapy of hepatitis B and D, exerts its function through inhibition of the hepatic bile acid transporter Na+-taurocholate cotransporting polypeptide (NTCP). In order to assess a myrcludex B-induced metabolomic response in humans, LC-MS-based monitoring of endogenous metabolites was performed in healthy individuals before and during treatment with Myrcludex B. Plasma samples were collected from healthy volunteers participating in a first-in-man phase I trial to evaluate safety, tolerability, and pharmacokinetics of single ascending doses of the NTCP inhibitor myrcludex B. Using quadrupole time-of-flight mass spectrometry coupled to reversed-phase chromatography (LC-QTOF-MS) a set of known NTCP substrates (bile acids) was quantified by targeted metabolomics. Protein precipitation was performed in the presence of deuterium-labeled internal standards which allowed absolute bile acid quantification in low amounts of plasma. The method was validated according to FDA guidelines and applied to monitor the effect of myrcludex B treatment on plasma bile acid level. Moreover, to investigate the effect of NTCP inhibition on global plasma metabolism, a non-targeted metabolomics approach was performed. Reversed-phase chromatography enabled the baseline separation of 15 human bile acid species which could be readily detected by accurate mass analysis in negative ion mode. Dynamic quantification of the targeted bile acid assay was achieved in the range from 7.8 nM to 10000 nM

depending on the analyzed species. Intraday and interday accuracy and precision were in the 15% tolerance range for all analytes. Recoveries and matrix effects were between 65-83% and 39-104%, respectively. Mean basal level of bile acids ranged from 15 nM tauroursodeoxycholic acid (TUDCA) to 1321 nM glycochenodeoxycholic acid (GCDCA) in plasma samples from healthy volunteers independent of a drug-induced NTCP inhibition. Myrcludex-induced NTCP inhibition resulted in a significant elevation of glycine- and taurine-conjugated bile acids after treatment whereas non-conjugated species were affected only partially. Investigation of plasma samples by a non-targeted approach confirmed the observed effects on bile acid homeostasis and revealed further, yet unidentified, metabolic features that are affected upon myrcludex B treatment. Metabolic alterations induced by a novel first-in-class entry inhibitor for the treatment of hepatitis B in humans.

POSTER 454

Metabolomics-Based Standardization in Quality Control of Human Plasma Samples

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Successful research in the healthcare area depends on high quality samples generated by standard sample collection methodologies. The quality of these biospecimens is impacted by pre-analytical processing steps that confound the analytical results and decrease the credibility of the research outcomes. Highest sample quality is particularly important in all projects which involve –OMICS studies or a systems biology approach. Metabolomics is a well-suited technology to support the identification of technical biomarkers for the quality assessment of biobank samples due to its high sensitivity plus the broad coverage of physiological and chemical processes. Human EDTA plasma samples obtained after applying defined pre-analytical confounding factors (e.g. wrong tube type, blood- and plasma storage, freeze-thaw cycles) were subjected to mass-spectrometry based metabolomics or to a targeted assay developed to control for such pre-analytical confounders. Pre-analytical confounders resulted in significant and reproducible changes of the human plasma metabolome with blood storage having the highest impact. The quality control assay detected plasma samples of a poor pre-analytical quality due to prolonged storage of blood or plasma or incubation at improper temperatures with a high sensitivity and specificity. Additionally, a sample type check allows to distinguish EDTA plasma from citrate or heparin plasma and serum. A score is calculated for each sample that indicates its pre-analytical quality. High-level result interpretation of -omics studies requires a comprehensive knowledge of the impact of the pre-analytical phase on the results and their underlying physiological and chemical mechanisms. The newly developed assay enables pharmaceutical R&D, clinical research organizations, and biobanks to better understand the actual condition of human plasma samples, efficiently monitor SOP compliance in multicenter trials, deliver superior quality samples, and support evidence-based decisions for sample selection. A novel metabolomics-based profiling assay assessing quality control of human EDTA plasma samples was developed.

POSTER 455

Current Trends in NIH Funding for Metabolomics Research in Epidemiologic Studies

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Metabolomics is emerging as an area of interest in the field of epidemiology that has significant potential

to evaluate the effects of nutritional, environmental, and pharmaceutical exposures; conduct risk assessments; predict disease development; and diagnose diseases. To understand the current funding trends, we evaluated the National Institutes of Health (NIH)-supported research grant awards to identify trends in metabolomics research. The objective of this study is to summarize metabolomics studies with an epidemiologic study design component that are currently funded in the NIH's grant portfolio. NIH-supported grants related to metabolomics and epidemiology that were considered active (funded) as of February 9, 2015 were included in the portfolio analysis. R24/U24 mechanisms and Center support grants were excluded from the analysis. The portfolio was analyzed using NIH's Query View Report (QVR). Search terms included metabolome, metabonome, metabolomic(s), metabonomic(s), metabolic profile, metabolite profile, metabolic signature, glycomic(s), and lipidomic(s). Criteria for inclusion in the analysis were: a) the focus is metabolomics, b) study involves human subjects, c) study must include an epidemiology component, and d) study has at least 100 cases and 100 controls. Analyses include basic descriptive statistics examining data coded by technology platform, approach, disease phenotype, biospecimen type, exposure, study design, and population examined. The initial term search identified 473 grants. After applying the inclusion criteria, 102 grants remained for further analysis. Preliminary analysis of these 102 grants indicates that the R01 funding mechanism was the most common (n = 49); however, several other funding mechanisms were also identified for these active awards, including training grants (K01, K08, K23), research program projects (P01, P20, P41), other research grants (R21, R33), and collaborative agreements (U01, U19, U54, UM1). Identifying the NIH Institutes and Centers (ICs) that primarily fund metabolomics grants with an epidemiology component may provide insight into the disease phenotypes that are mainly being investigated. Currently, National Institute of Diabetes and Digestive and Kidney Diseases (n=23), the National Heart, Lung, and Blood Institute (n = 21), and National Cancer Institute (n = 20) are the three Institutes that hold the most grants in this scientific area. The remaining grants are funded by 12 of the other ICs; therefore, approximately 56% of the 27 ICs are currently funding metabolomics research with an epidemiology component. Additional descriptive analyses investigating active grants as a function of technology platform, approach (i.e., targeted, semi-targeted, untargeted), disease state, biospecimen type, exposure, epidemiology study design, and population examined will help inform the field of current opportunities and challenges. This descriptive analysis will provide important insight into the current trends in metabolomics research with an epidemiologic study design component.

POSTER 456

Big data versus clinical judgment: can metabolomics overcome the translational gap?

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In the era of big data, metabolomics plays a central role in paradigms like translational research and precision medicine, which emphasize that the collection and analysis of data will improve the objectivity of disease diagnosis and treatment. As translational metabolomics research intersects with long-standing clinical practices, it has the potential to transform how clinicians think about and approach disease. But translational research faces challenges that are not only technical, but also social: the differing knowledge and priorities of researchers and clinicians, and the different domains and formats of molecular information and clinical data. Consequently, this presentation examines how and if big data can overcome the challenges inherent in the "translational gap" between metabolomics research from the laboratory to the clinic. This presentation is based on five years of qualitative social scientific research in the field of metabolomics, and is part of an NSF-funded project titled "What is metabolism after big data." In addition to participant observation and a systematic literature review, more than 50 in-depth interviews lasting 2 or more hours were used to collect data about the technologies, research goals, and challenges faced by metabolomics researchers. Data was collected from researchers at a number of UK Institutions, including Imperial College London and the European Bioinformatics Institute, with additional data collection planned at the UCLA Metabolomics Core and the West Coast Metabolomics Centre. The data were analyzed qualitatively using thematic coding with NVivo, as well as case studies analysis. To examine how and if big data can overcome the "translational gap," three core assumptions embedded within translational metabolomics research are analyzed. 1) Data is more objective than clinical judgment: Similar to clinical practice, there are many sources of uncertainty in metabolomics research. These include the challenges of (a) separating the metabolic activity of organisms from food,

drugs, and environments; (b) understanding individual and population variability; (c) interpreting biochemical in relation to known pathways and health outcomes. Until such challenges can be overcome, metabolomics data cannot be considered more objective than clinical judgment. 2) More data will lead to better health outcomes: Data pervade clinical and laboratory environments, such that translational research involves struggles to create, shape, and move data between laboratories and clinics. What counts as "data" is not the same for clinicians and researchers. Unless it can be integrated with clinical data, the collection and analysis of molecular data poses more of a problem than a solution. 3) Data can replace clinical judgment: Although data is said to overcome the shortcomings of clinical practice, it does not—and cannot—exist independently of human judgment. The design, collection, and analysis of data involve decisions and values. Big data displaces rather than replaces human judgment, locating it in research practices rather than patient interactions. Conclusions: To more effectively integrate laboratory research and clinical practice, emphasis should be placed on improving metabolomics' capacity not only to generate, standardize, and organize data, but also to make sense of complex datasets in relation to clinical outcomes. What is needed are more effective ways of harmonizing (1) different types and understandings of data, and (2) different forms of expertise. However, these should be developed in ways that do not prioritize big data over clinical practice, and which strive to "keep the human in the loop." This is the first (and to the author's knowledge, only) social scientific study of the emerging field of metabolomics research.

POSTER 457

Skeletal muscle metabolic and lipidomic networks regulated by the lipin 1 phosphatidate phosphatase, and MALDI MSI visualization

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The absence of the Lpin1 gene product, which decreases phosphatidic acid (PA) conversion to diacylglycerol (DAG), and prevents adipose tissue triglyceride formation, resulting in lipodystrophy. We previously showed (Xu et al Diabetes 2006) that the lipin 1 homozygous deficient fatty liver dystrophy (fld-/-) mouse model had dysregulated fuel switching, using glucose excessively in the post-prandial state, and fatty acids excessively in the re-fed state. We hypothesized the excess PA levels seen in the absence of lipin 1 would result in the conversion of excess PA to mitochondrial cardiolipin (CL), and that dysregulated fasted/re-fed changes in DAG and CL would occur influencing skeletal muscle fld-/- metabolic pathways, as DAG can influence insulin resistance, and CL aid the function of OXPHOS complexes BALB/c-WT and fld+/- and fld-/- mice were generated from fld+/- matings and assessed between 5-6 months of age. Freeze clamped (for LC/MS) and whole flash frozen (for MALDI) gastrocnemius muscle (GAS) was harvested in the overnight fasted vs. overnight fasted and 5 hour re-fed states. For MALDI, soleus (SOL), and extensor digitorum longus (EDL) as well as GAS were flash frozen in liquid nitrogen. serial sections were used for MALDI Thermo LTQ XL MSI (matrix 0.5M 2,5-dihydroxybenzoic acid (DHB) in methanol or 10 mg/mL 9-aminoacridine (9AA) in 70:30 (v:v) ethanol:water), and SDH, COX and ATP stains Targeted LC/MS for glycolytic and TCA metabolites were assessed using a Waters TQ MS, and lipidomic analyses were done with a Thermo Q Exactive. DAG species were decreased, and CL was increased, in either the fasted or re-fed states for fld-/- vs. WT or fld+/- GAS, expected since PA->DAG conversion is inhibited with lipin absence, causing PA diversion to CL formation. For fld-/- vs WT GAS between the fasted and re-fed states, greater increases were seen in glucose-6-P, fumarate and succinate, while glycerol-3-P and pyruvate showed equal increases. Insulin was 20 fold increased between the fasted and re-fed states for fld-/- mice, as opposed to 2-fold for WT or fld+/- mice. Increases in glycolytic intermediates between the fasted and re-fed states were not seen for fld+/- mice, with the exception of fructose-1,6-bisphosphate (F-1,6-BP), which was 4-fold increased between the fasted and re-fed state for fld+/- mice. No changes in F-1,6-BP was seen between fasted or re-fed GAS for fld-/- vs WT, but F-1,6-BP was 2-fold high in fasted GAS for fld-/- vs fld+/-. MALDI was done for GAS to examine glycolytic metabolite changes. Visualization of metabolic pathways using

MALDI showed increased levels of F-1,6-BP across fld-/- vs fld+/- GAS in the post-prandial state. . ATP, ADP and AMP as well as inosine-phosphate (IMP) was detected, and tandem mass spectrometry was used to confirm their identities. We have found that when muscles are sectioned and coated rapidly, analysis of nucleosides are detectable. While ATP, ADP and AMP showed small increases in intensities across GAS for fld-/- vs fld+/- muscles, IMP was seen to be elevated across GAS fld-/- vs fld+/- muscles. In conclusion, insulin resistance may result in increased glycolysis due to overshooting of the insulin re-feeding response. In this case of lipin absence, where CL is elevated, which can facilitate OXPHOS function, the input of glycolytic flux into the TCA cycle to generate energy may be increased, visualized by MALDI examination of nucleoside phosphates. MALDI can be a useful tool for visualizing skeletal muscle metabolic pathways associated with dysregulated fuel switching.

POSTER 460

Imaging of prostate cancer tissue biomarkers by MCAEF (matrix coating assisted by an electric field) – laser desorption/ionization mass spectrometry

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Prostate cancer is one of the most common malignancies worldwide. Because its pathogenesis has not yet been fully deciphered, the discovery of new and more reliable biomarkers to stratify its onset and progression is of great importance. Tissue imaging by matrix-assisted laser desorption/ionization mass spectrometry (MALDI-MS) is a promising technique to assist in the comprehensive and detailed analysis of the spatial distributions of ionizable molecules in situ. In this work, we applied a newly-developed matrix coating technique -- matrix coating assisted by an electric field (MCAEF), in combination with positive (+) and negative (-) ion LDI-MS, to more comprehensively profile the endogenous compounds which could clearly differentiate between the cancerous and non-cancerous regions of human prostate tissue. Three human prostate cancer (stage II) tissues were cryosectioned into 12-μm thick slices and thaw-mounted onto ITO-coated glass microscope slides. The tissue sections were coated with MALDI matrices, including quercetin, 9-aminoacridine (9-AA), and sinapinic acid (SA), under an optimized static electric field (i.e., MCAEF). A 12-Telsa quadrupole-Fourier transform ion cyclotron resonance (FTICR) instrument and a MALDI-time-of-fight (TOF)/TOF instrument were used for metabolite and protein imaging, respectively, at 200-μm pixel resolution. Bruker FlexImaging was used to reconstruct the ion maps. The metabolites were identified by accurate mass measurements, MALDI- or LC-MS/MS, and querying the metabolome databases (METLIN and LIPID MAPS); the proteins were identified by top-down or bottom-up LC-MS/MS analyses followed by database searching against the Uniprot-Swissprot and Uniprot-Trembl databases. The use of MCAEF (Wang, et al. Chem. Sci., 2015) significantly enhanced LDI-MS detection of both metabolites and proteins in the tissue sections and resulted in an approximately 90% increase in the number of the imaged biomolecules, as compared to electric field-free matrix coating. Using quercetin and 9-AA as the two complementary MALDI matrices for (+) and (-) ion detection, tissue imaging by MCAEF-LDI/FTICR MS led to the imaging and localization of a total of 864 metabolites (813 lipids and 51 primary metabolites). 147 compounds were uniquely detected in the non-cancerous cell region and 264 compounds were uniquely detected in the cancerous cell region. Of the other 453 imaged metabolites, 51 showed distinct distributions ($p<0.01$, t-test) between the cancerous and non-cancerous regions. Overall, 53% of the detected metabolites showed significantly different distributions between the two regions. The use of SA as the matrix with MCAEF-LDI/TOF MS resulted in the successful detection and imaging of 242 peptide and protein signals between m/z 3500 to 37500, with 64 species being uniquely detected in the cancerous region, while no unique peptides or proteins were observed in the non-cancerous region. Of the 178 species detected in both cellular regions, 27 species (including prostate-specific antigen, tumor protein D52, and a fragment of mitogen-activated protein kinase/extracellular signal-regulated kinase kinase kinase 2) showed differential distributions ($p<0.01$, t-test). Of these up- or down-regulated proteins, the distributions of apolipoprotein C-I, S100 A6, and S100 A8 were verified by immunohistological staining. In summary, the spatial locations and abundances of these biomolecules indicated significant molecular alterations in the prostate cancer cells. This study

resulted in the largest number of endogenous biomolecules in prostate cancer imaged thus far by MALDI-MS and shows the great potential of MCAEF-LDI MS imaging for enhanced in situ detection of biomarker candidates in cancer tissue. MCAEF was shown to be a robust technique for enhanced MS imaging of biomarker candidates for prostate cancer.

POSTER 461

Visualization of metabolic images of Langerhans islets by ultra-high resolution LDI - MS imaging using Au nanolayers

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Mass spectrometry imaging (MSI) allows the mapping of chemical compounds on a tissue section. The most widely used ionization in MSI is Laser Desorption/Ionization (LDI) where tissue is scanned forming an image in which each pixel corresponds to a mass spectrum. Conventional LDI methods for MSI are based on spraying an organic matrix, reducing the effective lateral resolution and producing strong background noise inherent to the matrix in low mass range.Here, we obtained images from rat pancreas by means of a valuable matrix-free LDI based on the deposition of gold nanoparticles straight over the tissue. With the aim of identifying different functional parts of the tissue, multivariate analysis algorithms have been applied leading to the generation of molecular images. Rat pancreas tissues were sliced into 10 µm sections using a cryostat and directly placed on indium tin oxide (ITO)-coated glass slides. Next, a 3 nm layer of gold nanoparticles was deposited over the tissue using a sputtering system running in RF mode. The MS spectra were acquired with a Bruker UltrafleXtreme MALDI-TOF instrument. The instrument has been adjusted with a raster step down to 10 µm, a total accumulation of 500 laser shots per pixel, a laser frequency of 2 kHz and a laser attenuation of 60%. After the acquisition, data was exported to XMASS format and converted to a custom format to perform data processing using in-house developed software, consisting in several preprocessing steps and image segmentation routines. The method presented here, was able to acquire MS images in a wide low-mass range (< 1000 Da) exhibiting a negligible background signal from gold nanoparticles. Sputtering is a quick, flexible and easy to use deposition technique used to grow highly controlled homogeneous layers, needed for avoiding differences in ionization efficiency between pixels. Further on, ultra-high lateral resolution (< 10 µm) is achieved, due to the absence of lateral compound diffusion. In order to quantify the lateral resolution, we have imaged some small regions of tissue using various raster sizes (from 10 to 100 µm) concluding that the limiting factor of resolution came from the instrumental laser focalization and not from the gold layer itself. Besides the good characteristics of our gold layer, another advantage is the reduced time of sample preparation, less than 15 minutes in contraposition of one hour long of conventional organic matrix deposition procedures.In order to study the quality of the method, the MS image obtained from some pancreas of healthy and diabetic rats has been processed using several algorithms. First, images of some ions have been reconstructed and some spectra have been examined to evaluate the ionization efficiency. Secondly, an image of total ion count (TIC) was produced exhibiting an intensity variation uncorrelated with deposition method. After the application of the common preprocessing steps, several segmentation algorithms, like principal component analysis (PCA), have been used for clustering purposes, producing images where the Langerhans islets could be clearly differentiated from exocrine and adipose tissue regions. The ultra-high lateral resolution has been essential to study the molecular composition, as well as the density and distribution of Langerhans islets, making the Au nanolayer MSI a powerful tool for the study of diabetes. Sputtered Au based MS imaging is an ideal method for metabolite imaging presenting high lateral resolution and negligible background noise.

POSTER 462

NEW WO3 SOLID–STATE SURFACES FOR LASER DESORPTION/IONIZATION MASS SPECTROMETRY FOR HIGH THROUGHPUT METABOLOMICS

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Matrix Assisted Laser desorption/ionization mass spectrometry (MALDI-MS) is commonly used in proteomics because of the easy sample preparation, and fast analysis. However, the applicability of this technique in metabolomics is limited due to the presence of interference peaks in the low mass range (<1000 Da) inherent to the organic matrices needed for compound ionization. To overcome this, in the recent years solid-state surfaces have been introduced for LDI-MS metabolomics applications that allow the ionization of a wide mass range of metabolites with reduced background noise. Here, we introduce WO3-based surfaces as a valuable alternative for LDI-MS metabolomics applications. Performance of the WO3-surfaces was tested in a biological study and compared with nanostructure initiator mass spectrometry (NIMS), a well-established matrix-free platform. Three different WO3-based surfaces were fabricated for this study: two types of anodized layers of WO3 on silicon, one anodized with tartaric acid (WO3) and another also with a layer of bis(heptadecafluoro-1,1,2,2-tetrahydrodecyl)tetramethylsiloxane (WO3-BisF17) and one NIMS functionalized with WO3 nanoparticles (NIMS-WO3) deposited by sputtering, to explore the changes in NIMS properties when functionalized with WO3 nanoparticles. As a reference, we have compared the performance of these WO3 surfaces with a standard NIMS. The performance of the surfaces was checked using 20 standard compounds (from m/z 90 to 800, including amino acids, nucleotides and lipids). The performance of the four surfaces was tested in a metabolomics study, consisting in the analysis of urine from rats before and after 3,4-Metilendioximetamfetamina (MDMA) administration. As commented above, background noise is one of the main limitations for the applicability of LDI-MS in metabolomics. The background of WO3 surfaces developed in this study presented significantly less peaks with lower intensities than those found in the reference surface (NIMS). In this respect, WO3-BisF17 and NIMS-WO3 presented less than 120 background peaks with median intensities under 200 (NIMS more than 500 peaks with 1000 median intensities). Regarding the performance of the surfaces with the metabolites standards, NIMS-WO3 and WO3-BisF17 were able to detect the lipids, with enhanced sensitivity in the NIMS-WO3 surface. The performance of the surfaces with most of the polar standards (e.g. amino acids and short chain acids) was similar, with average s/n ratios between 100 and 1000. Exception was, for instance, folic acid, which was only detected with the WO3 and WO3-BisF17 surfaces. The biological study was conducted by analyzing the urine from 12 rats treated with MDMA. Previous to the LDI-MS analysis, urines were just diluted to a factor of 1:10. All the studied surfaces showed peaks inherent to the urine samples, including creatinine. The changes in the urine metabolome were determined by comparing the basal urine with the rat urine 24 hours after the intervention, finding statistical significant differences before and after the treatment for all surfaces. These results were partially validated by NMR. In conclusion, the WO3-based surfaces developed here allow the use of LDI-MS for metabolomics studies, offering a reliable alternative to NIMS with less background noise facilitating the correct peak detection, and better sensitivity for some metabolites. Furthermore, the fabrication of the WO3 on silicon surfaces is rapid (less than 30 min) and avoids the use of a highly corrosive acid (HF) needed on NIMS elaboration. New WO3 solid-state surfaces for LDI-MS metabolomics with lower background noise, safer fabrication process and enhanced results to NIMS surfaces.

POSTER 463

Direct analysis of metabolites and enzymatic activity in cell lysates or culture media with mass spectrometry imaging

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Nanostructure-Initiator Mass Spectrometry (NIMS) has been used for metabolite analysis in a diversity of applications because of its high sensitivity and low background. However, metabolites in complex sample matrices, e.g. cell lysates or culture media, need to be pre-treated in order to simplify the samples, which reduces either throughput or general applicability. Here we investigate the use of NIMS for the screening of cell lysates or culture media on metabolites and enzymatic activity in a facile and high throughput manner. We aim to use the hydrophobic NIMS chip surface to separate metabolites from inhibitory compounds. Small sample volumes (1-10nl) will directly be applied onto the NIMS chip surface using acoustic disposition, which allows analysis 10,000 samples per day. Sample preparation Either culture media or cell lysates will be mixed with different solvents (e.g. methanol, 2-propanol or acetonitrile) at different ratio's and with/without additives (e.g. TFA or FA) in either 384- or 1256-well plates. Cellular debris will be removed using centrifugation. Acoustic disposition and NIMS analysis The samples will be spotted onto NIMS chips using an acoustic printer (EDC ATS-100) at deposition volumes of 1-10 nL. An AB Sciex TOF/TOF 5800 MALDI mass spectrometry system combined with MALDI MSI 4800 imaging software will be used for mass spectrum imaging. OpenMSI will be used for the analysis of the obtained image spectra. In this study we want to make use of the hydrophobic surface of the NIMS chip surface by using it as stationary phase for separation of metabolites from inhibitory compounds (mainly salts). Separation of the metabolites from salts is achieved due to differences in attraction to the stationary phase and solubility in the used solvents. By using different solvent mixtures, various degrees of separation of metabolites from salt was observed using NIMS. Using the separation power of the NMS chip surface, we want explore screening of culture medium and cell lysates on metabolites. We aim to screen several different bacterial strains and culture media in order to determine the maximal number of detectable metabolites. Furthermore, the effect of the solvents, solvent ratio's and additives on the detected metabolites will further investigated. We want to determine if specific sets of metabolites require specific solvent conditions. In addition, we also want to explore screening of bacterial lysates on enzymatic activity. We aim to screen both recombinantly expressed and endogenous enzymes against a small library of chemicals. Initially, we will screen two N- and S-methyltransferases and this could be further extended to other enzyme classes. Analysis of metabolites and/or enzymatic activity in cell lysates and media in facile high throughput manner using NIMS

POSTER 464

Label-free Raman imaging of living mammalian cells - a valuable new tool for investigating complex cellular systems.

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A current area of research that is gaining increasing interest is the field of single cell analysis (SCA). Raman mapping offers a powerful opportunity for the analysis of single cells due to its high spatial resolution (< 1 μm), speed of data acquisition and non-invasive and non-destructive nature. Additionally, as water is a weak Raman scatterer, spectral contributions pertaining from aqueous cell content is minimal. Raman mapping is capable of providing vast amounts of spatially resolved biochemical information regarding the composition of cellular and sub-cellular components. In our laboratories we have recently established the capability to undertake 'true' live cell Raman microspectroscopy by coupling a Raman microscope with a near infrared (NIR) laser source and an in situ microscope incubator. All Raman spectra were collected using a Raman system (inVia, Renishaw plc, UK) coupled to a 785 nm spot focus laser, with the addition of a commercially available incubator (Okolab, Italy) programmed to provide sufficient atmospheric conditions for optimal cell growth (5% CO_2 and 37 °C). Two model cell systems were used in this work, an immortalized human keratinocyte cell line (HaCaT) and a genetically engineered human beta cell line (EndoC-βH1). Dithranol (2 μM) was applied to the HaCaT cells for 24 h and the uptake and localization of the drug was monitored in real-time. The EndoC- βH1 cells were

stimulated with glucose (low (5 mM) and high (25 mM)) and the stimulated insulin secretion monitored. This work clearly documents the exciting applicability of Raman Spectroscopy for the analysis of 'truly' live mammalian cells, that is to say that the cells are analysed whilst actively growing in their normal culture medium and thus metabolizing in a normal manner. The analysis of live cells overcomes the inherent issues associated with the analysis of fixed cells that only present a single 'snapshot' of cellular metabolism. Additionally, our analysis has been conducted label-free without the requirement of adding tags to the system (nanoparticle or fluorescent). Dithranol is a drug that is highly amenable to Raman analysis as it has a significant Raman scatter. We are able to clearly observe the uptake of dithranol (at a clinically relevant concentration) in real-time and further monitor its localization within the cells. The analysis of Raman maps collected from a number of cells demonstrated a variable uptake mechanism and localisation pattern within the cells, which is perhaps not surprising due to the heterogeneous nature of a cell population. Glucose stimulated insulin secretion is a response mechanism that is of great interest in the search for cellular-based therapies for the treatment of diabetes. The current method for this detection is via ELISA. Our hypothesis is that we can use Raman spectroscopy to monitor the effect of stimulation on the cell and also use Raman to detect insulin secretion into the culture medium in real-time. Thus, this technique could be applied to the analysis of multiple model cell systems and various investigations focusing on areas of research including for example, the mechanism of cell differentiation or cell death or the response of cells to chemical perturbations such as drug molecules or extreme culture conditions. A novel, label-free and non-invasive approach for the analysis of cellular perturbations in real-time with appropriate case studies presented.

POSTER 465

The potential of spatially-resolved, LAESI-MS based metabolic mapping in plants

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Plant tissues are a tremendously rich source of highly diverse metabolites, many of which have as yet unknown function. We are also aware that many such so-called 'secondary' metabolites are not evenly distributed throughout the plant but rather, have very strong organ, tissue or even cell-based localization. Knowledge of where exactly plant metabolites are accumulated and their general spatial heterogeneity would greatly help us build a more detailed picture of pathway activity and control. A key limitation to extract-based metabolomics analyses is that spatial heterogeneity is generally lost as we need to generate representative samples using significant amounts of pooled tissues. In situ metabolite imaging-based approaches have the potential to overcome this loss of spatial resolution. Plant tissues are a tremendously rich source of highly diverse metabolites, many of which have as yet unknown function. We are also aware that many such so-called 'secondary' metabolites are not evenly distributed throughout the plant but rather, have very strong organ, tissue or even cell-based localization. Knowledge of where exactly plant metabolites are accumulated and their general spatial heterogeneity would greatly help us build a more detailed picture of pathway activity and control. A key limitation to extract-based metabolomics analyses is that spatial heterogeneity is generally lost as we need to generate representative samples using significant amounts of pooled tissues. In situ metabolite imaging-based approaches have the potential to overcome this loss of spatial resolution. As LAESI does not require either a vacuum or an ectopically-applied chemical matrix it is possible to perform all analyses in situ and under ambient conditions. Using variegated Phalaenopsis petals for a positive proof of concept, employing LAESI MSI, it was readily possible to confirm the degree of spatial resolution of the imaging system by matching anthocyanin metabolic maps with their already visible, spotted distribution across the petal surface. Additional maps made of other, invisible phenolic compounds revealed some to match almost exactly the distribution of anthocyanins (co-localization) while others were found to localise exclusively in the white anthocyanin-free regions. Considering the close biochemical pathway relationships between the metabolites concerned, it is clear that localisation of many metabolites is under strict cellular control. Using tomato leaves it has also been possible to demonstrate significant heterogeneity in alkaloid distribution in plants grown under standard control conditions. Following infection

of susceptible tomato leaves with C. fulvum, clear localised perturbations in alkaloid content were observed as a result of the presence of the pathogen. In the presentation attention will also be drawn to some of the drawbacks and limitations of both data generation and analysis and point to some of the potential pitfalls when using such an approach for comparative metabolomics. Applying the technology and interpretation of the data is not straightforward and this therefore, demands further research and dedicated tools to transform this approach into a robust metabolite localization method down to the tissue and hopefully eventually also, the cell level. MS – based metabolite imaging is an upcoming field; LAESI approaches are of great interest for analysing living plant material.

POSTER 466

Understanding metabolic processes in tissues using DESI- and MALDI-MSI – Competition or Complementarity?

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Desorption electrospray ionisation imaging mass spectrometry (DESI-MSI) is a recent technique that is rapidly gaining popularity in spatially resolved metabolomic analyses of tissue sections. Matrix assisted laser desorption ionisation (MALDI) is a substantially more established technique in this area than DESI and is thus a useful benchmark for evaluating DESI performance. We show that DESI can not only compete with MALDI in lipid imaging in human tissue samples but also facilitates access to 3molecules which are difficult to analyse with MALDI due to matrix interference, in particular to low molecular weight molecules, such as endogenous metabolites of energy-producing pathways. To compare the two methods' performance for differentiating tissue types, sections of human colorectal cancer metastasis embedded in healthy liver tissue were analysed with both methods in negative ion mode. Consecutive 14 um thick sections of each sample were analysed. A preliminary analysis of the data was performed, where 20 pixels were selected from both tumour and healthy tissue for each method and subjected to multivariate analysis (Principal Component Analysis, PCA). A section of the same tissue was submitted to further imaging with a focus on the mass range from 50 to 500 m/z, in order to investigate small endogenous metabolites. A further section from the same sample was washed with chloroform to remove lipids and analysed for endogenous metabolites. Results of the PCA show that although the DESI and MALDI spectra are markedly different and separate out in principal component space, both analytical methods are capable of separating the healthy from the cancerous tissue, based on their metabolic profile. Examination of the PC loadings shows that the separation is based on similar spectral features, i.e. that DESI and MALDI pick up on the same set of molecules responsible for differentiation. The two tissue types compared in this preliminary study were markedly different biologically (colorectal cancer versus healthy liver tissue). The next step will be to compare tissues that are more similar, such as colorectal cancer versus healthy colorectal tissue or different stages of cancer and show that DESI can compete with or even outperform MALDI. The lower mass region of DESI spectra of tissue samples is usually dominated by free fatty acids. However, this mass range is not obscured by matrix peaks like it often is in MALDI. Chloroform washing of the section is an efficient method for lipid removal (incl. fatty acids) and can improve detection of other small endogenous metabolites using DESI even further. Metabolites found to be elevated in cancerous versus healthy tissue included, among others, glutamate, aspartate, and taurine. Glyceryl-phosphorylethanolamine was found to be concentrated in the healthy liver tissue. This molecule is a breakdown product of phosphatidylethanolamines and acts as a growth stimulant for hepatocytes. Identification and correlation of further endogenous metabolites is likely to provide further insights into metabolic differences between the two tissue types examined. DESI and MALDI-MSI are compared for lipid fingerprinting and low molecular weight metabolomics analysis of human cancer tissue.

POSTER 468

Metabolic profiling of Drosophila melanogaster metamorphosis

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As a holometabolous insect, the development of Drosophila has to undergo metamorphosis which requires a lot of energy to reconstruct the body into the adult form. Previous studies shown that fat body, accumulated during the extensive feeding in larval stage, is an important energy reservoir accounted for more than 80% of total metabolism to support the animal through the subsequent pupation. However, how the flies use this source to generate energy is not clearly understood. Since Drosophila melanogaster has been widely used as a model organism, the deeper knowledge on its development is required. Therefore, the aim of this study is to elucidate the metabolism during Drosophila metamorphosis by performing metabolic profiling with GC/MS and LC/MS. Samples were collected during the metamorphosis (1st-15th pupation stage) of Canton S, the Drosophila wild type strain, according to the morphology described by Bainbridge et al. in 1981. Ten pupae were used in each sample and the experiment was conducted with 5 biological replicates. Then, we performed a combination of GC/MS (GCMS- QP 2010 Ultra, Shimadzu) and LC/MS (Nexera UHPLC coupled with LCMS 8030 Plus, Shimadzu) in the same extracts for metabolic profiling. The extraction solvent consisting of methanol/water/chloroform (2.5:1:1) was used to extract a wide range of low molecular hydrophilic metabolites. After peak annotation, we normalized the data to total ion chromatogram and the obtained data were applied to multivariate analysis. An instantaneous snapshot of the physiology of Drosophila during metamorphosis was observed by employing non-targeted GC/MS-based and targeted LC/MS-based metabolic profiling. The detected metabolites related to the central metabolic pathway including the metabolism of amino acid, sugar and nucleic acid, TCA cycle and urea cycle. The results from hierarchical clustering analysis and principal component analysis have shown that distinct metabolites are activated and intensely linked with different stages of pupation. Moreover, we were also able to construct a prediction model for pupal stages based on metabolic profile by using partial least square projection to the latent structure. From this model, the important metabolites together with its correlation with these developmental stages will be proposed. Finally, by applying pathway enhancement analysis, the general view on precise pathways and deeper discussion on their roles during metamorphosis of Drosophila will be provided. The new insight into the energy metabolism of Drosophila metamorphosis was elucidated.

POSTER 469

A core metabolic enzyme is responsible for phosphine resistance and fundamental metabolic regulation.

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Phosphine (PH3) is a small redox-active gas that is used to protect global grain reserves, which are threatened by the emergence of phosphine resistance in pest insects. Despite its importance, little is known about the toxic action of PH3 or the resistance mechanisms. The nematode Caenorhabditis elegans is also vulnerable to phosphine, can be easily studied, and is thus an excellent model system for phosphine resistance in insects. The key resistance factor, dihydrolipoamide dehydrogenase (DLDH) is implicated in lifespan determination as well as multiple ageing-linked diseases in humans. We have created a phosphine resistance mutation in Caenorhabditis elegans that also causes post-reproductive lifespan extension by 30%. This allowed us to use C. elegans as a model organism to investigate and characterise the mechanisms of phosphine toxicity and resistance employing a combination of genomics and NMR-based metabolomics. Determining the sources of metabolic variation between resistant and susceptible strains and characterisation of the corresponding compounds allowed us identify the metabolic pathways affected by phosphine poisoning and phosphine resistance. that corresponded to the dihydrolipoamide dehydrogenase gene that had been identified by genetic mapping. Extensive homology modelling of the DLDH enzyme revealed the likely structural and functional basis of resistance. PLS-DA

analysis of the metabolite profiles show four clusters, which allow to distinguish between (1) genotype-differences between wild-type and phosphine-resistant strain, and (2) phosphine-exposure effects. Furthermore, the phosphine response of the resistant C. elegans is (a) less pronounced than, and (b) orthogonal to the phosphine response of the wild-type, indicating not only that the mutant is less affected than the wild-type, but that the metabolites of these two strains changed differently in response to phosphine. Both are classical hallmarks of resistance. Indeed, the resistant strain behaves in air as if it already had been exposed to phosphine, exhibiting down-regulated metabolism and low oxygen consumption. We investigated whether hypoxic adaptation plays a role in phosphine resistance and found that hypoxia-adapted C. elegans strains are indeed partly phosphine resistant. Ultimately, we identified dihydrolipoamide dehydrogenase (DLDH) as the enzyme responsible for phosphine resistance. Homology modelling, based on the known structures of human and yeast DLDH, reveals that polymorphisms responsible for phosphine resistance fall in two separate classes in both pest insects (Rhyzopertha dominica and Tribolium castaneum) and in C. elegans: They either cluster around the redox-active catalytic disulfide, or they are located at the dimerisation interface of DLDH. DLDH is a core metabolic enzyme, central to metabolic regulation, and a new class of resistance factor for a redox-active metabolic toxin. It participates in four key steps of core metabolism, which are affected differently by phosphine exposure in mutant and wild-type animals. This mild pathway-preference can be explained based on the protein structure. The position of DLDH in the metabolic network makes it a likely candidate for a central regulator of metabolism. DLDH is also implicated in other biological processes, such as lifespan extension and hibernation, or the development of diseases such as cancer and Alzheimer's. This successful systems biology approach facilitates further characterisation of DLDH, an enzyme of profound importance to agriculture and human health.

POSTER 470

Life extending mild heat treatments induce long-term changes in metabolites associated with energy metabolism in Drosophila melanogaster

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Short bouts of mild stress early in life can affect many life-history-traits at later life-stages. Depending on the severity of the stress and the state of the individual experiencing the stress, the late-acting effects might be beneficial or detrimental. The beneficial effects probably occur when stressful conditions induce maintenance and repair pathways to an extent that exceeds the damage that the stress has caused. This is termed hormesis and among other effects it can result in increased general stress resistance and lifespan. As of yet little is known about the exact mechanisms underlying hormesis. We used NMR spectroscopy to investigate the long-term effects of repeated mild heat treatments (day three, six, and nine), previously shown to increase lifespan, on the metabolome of Drosophila melanogaster from 3 genetically independent lines. Five replicates of samples consisting of 50 males each were taken before heat treatment and from untreated (L) and treated (H) flies at day 19 and 35. Principal component analysis (PCA) was carried out on the full data using Simca 13.0. The number of significant PCs was assessed by leave one out cross validation. Metabolites affected by the hormesis treatment were identified using t-tests between H and L spectra at 19 or 35 days of age. The metabolome of the heat-treated flies is clearly distinguishable from control flies 10 days after the last bout of stress. 40% higher levels of alanine and lactate were measured. Glucose was also higher and glutamate lower, both by 10%. However, 26 days after the last bout of mild stress, no general effect of the heat treatments could be detected in the metabolome. The metabolite changes induced at day 19 by the hormetic treatment are indicative of a shift in the energy metabolism. Glucose is metabolized to pyruvate, which in turn can be transformed to acetyl CoA, lactate and alanine. Acetyl-CoA is synthesized by the pyruvate dehydrogenase complex and is usually further metabolized in the TCA cycle or used for fatty acid synthesis. Lactate is synthesized by lactate dehydrogenase in the cori cycle, and alanine by alanine transaminase in the alanine cycle using glutamate for the transamination. Glucose is regenerated from alanine and lactate in the fat body. Thus, to obtain the observed changes either the glucose intake has been increased, the need for acetyl-CoA is smaller, or there is a change in regulation of the energy metabolism. Metabolic reprogramming has previously been associated with the life extending effects of

dietary restriction. The metabolite changes indicated a change in energy metabolism, suggesting that hormesis is not only due to induced repair pathways.

POSTER 471

Redox state alteration as regulatory mechanism during oogenesis in D. melanogaster

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Reactive oxygen species (ROS) along redox homeostasis have emerged as regulators of aging, age-related diseases and cancer. ROS have detrimental effects for the cell: peroxidizing lipids and disrupting proteins and nucleic acids. Recently, this dogma has been challenged by studies suggesting a physiological role for ROS for signaling cascades and gene expression. A physiological increase in ROS, coincident with an increase in metabolic demand, also has been shown during oogenesis, as the specialized oocyte transforms to a totipotent cell capable of sustaining the rapid cell divisions of early embryogenesis. A direct role for ROS and redox state during this transition is hypothesized, when ROS could act as an environmental sensor fine-tuning the relationship between reproductive potential, age and environmental conditions. In Drosophila melanogaster the female-expressed thioredoxin gene, deadhead (dhd), is specifically required during oogenesis, and its absence leads to meiotic defects and a block in early development. Metabolomic profiling as well as redox sensitive GFP (roGFP) imaging in combination with the powerful genetic and biochemical tools offered by the Drosophila system will define the redox state changes during oogenesis and early embryogenesis. Preliminary data indicate a link between the meiotic cell cycle control system – the meiosis specific APC/C Cortex, and Dhd, suggesting tight regulation of Dhd protein levels and thus redox state during oogenesis. An in vitro protein destabilization assay is established to probe this regulatory mechanism in further detail. We investigate how redox regulates oogenesis, where ROS could serve to coordinate completion of oogenesis with developmental and/or environmental cues.

POSTER 472

Metabolomics approach to understand the effect of genotype and diet on metabolic profile of Drosophila.

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Over past decade, Drosophila has emerged as one of the important model organisms for evaluating various aspects of Metabolic Syndrome (MetS), a complex disease that increases the risk for heart disease and diabetes. The prevalence of MetS has been attributed to the westernized dietary habit and sedentary lifestyle. Our previous studies (Reed 2010 and 2014) have established, diet as one of the important contributors to metabolic phenotypes. However, lack of a comprehensive metabolic profile of Drosophila, has been a key shortcoming for employing this model for such analyses. In order to fill this information gap, we have employed a metabolomics approach to isolate and identify global metabolites in Drosophila larvae and classify key metabolites to analyze important differences in diet. Drosophila larvae consisting of 16 genotypes, representing two phenotypic groups for triglyceride storage, were fed on normal and high fat diet. The larvae samples were then analyzed using Liquid chromatography/Mass spectrometry (LC/MS) and Gas chromatography/ Mass spectrometry (GC/MS) to obtain the metabolite concentration data. Normalization and unit variance scaling of the concentration data were performed prior to the classification analysis using Random forest (RF), in MetaboAnalyst (v3.0). The important metabolites identified by random forest as key classifiers were subjected to Hierarchical clustering using Ward's algorithm. The spectral analysis detected a total of 350 metabolites, 270 of which have definitive chemical IDs and 80 being unknown, fulfilling our objective of expanding the metabolic repertoire of Drosophila. Further, RF analysis deemed 15 metabolites to be important classifiers between the two comparative diets, with fatty acids emerging as key classifiers for high fat diet. The cluster analysis further showed that the differing metabolites between two diets tend to cluster together irrespective of the

genotype and/or triglyceride phenotype, showing a direct effect of diet on the metabolic profile. Overall, the study was able to successfully employ a comprehensive classification model to illustrate diet as one of the important contributors to metabolic phenotypes. The study expands the global metabolic profile of Drosophila, additionally classifying specific fatty acids as key classifiers between two diets.

POSTER 473

Dietary effects on the association between metabolite and gene expression using eigenvector metabolite analysis

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Metabolic Syndrome (MetS) is a complex disease, which manifests symptoms including central obesity, insulin resistance, and elevated blood pressure. The occurrence of this disease, which leads to an increased risk of heart disease and type-2-diabetes, has been growing. Interactions between genetic and environmental effects (Westernized diet and sedentary lifestyle) promote MetS, and these effects also influence metabolite expression. In this study, we quantified the metabolomes of 20 genotypes of Drosophila melanogaster that were reared on four different diets. We clustered the metabolites based on correlations and recovered chemically related groups of metabolites within the clusters. The linkages between correlated metabolite clusters, gene expression, and MetS phenotypes were characterized using the first principal component of each cluster. These analyses demonstrated that some clusters of metabolites are significantly correlated with groups of genes enriched for physiological processes that utilize these metabolites. However, diet does not affect the expression patterns of all metabolites. Specific MetS phenotypes were also found to associate with specific metabolite clusters in a diet specific manner indicating context dependent mechanistic links between the MetS phenotypes and diet. We use innovative multivariate analysis methods to link metabolomic variation across genotypes and diets to gene expression and disease phenotypes.

POSTER 475

Combination of elemental and molecular mass spectrometry for rhizosphere metabolomics

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The understanding of rhizosphere processes is indispensable for optimizing plant performance in terms of yield, but also in the context of bioremediation strategies. Rhizosphere metabolomics aims at the analysis of the entire metabolome of the root-soil interface addressing root exudates, exudates of the root associated microbiome and fungi. The major target compounds are primary metabolites, e.g. organic acids and carbo-hydrates, as well as secondary metabolites (1). As a matter of fact, representative sampling and accurate analysis of root and soil bacterial exudates is extremely challenging. In this work we will present advanced sampling strategies and mass spectrometry based analytical approaches comprising metabolite profiling and non-targeted analysis.

References: (1) Zhang et al., Curr. Op. Biotech., 2015, 32, 136-142

Targeted quantitative analysis of organic acids was performed via LC-MS after derivatization by esterification with benzyl alcohol. Amino acids and phytosiderophores were quantified with GC-MS or LC-

MS/MS. Phytosiderophore-metal complexes were speciated by LC-ICPOSTER MS using a mixed-mode stationary phase. Non-targeted approaches were performed with high resolution accurate mass LC-TOFMS and GC-TOFMS systems. The developed molecular and elemental mass spectrometric methods were applied in two case studies. The first experiment aimed at the investigation of phytosiderophores, a group of root exudates released by grass species (Poaceae) for iron mobilization. We conducted a rhizotest experiment with different soils and wheat grown under normal and Fe-deficient conditions. A significant increase of root exudation and release of the phytosiderophore 2´- deoxymugineic acid was found for Fe-deficient wheat. Moreover, metal mobilization by 2´- deoxymugineic acid was investigated in different top soil extracts via quantification of metal-phytosiderophore complexes. The data revealed quantitative complexation of the extracted metal fractions. Non-targeted analysis was conducted in the context with microbiomes associated with Zn-accumulating Salix species. It is hypothesized that secondary metabolites, e.g. bacterial siderophores, are involved in metal mobilization. More specifically we analyzed soil extracts incubated with cell culture supernatants from bacteria isolated from Zn accumulating S. caprea. Statistical evaluation of the data sets revealed several putative metal mobilizing compounds. A portfolio of complementary mass spectrometric methods is presented showing the potential of metabolomics in rhizosphere research

POSTER 476

Monitoring of Bacterial Soil Organic Matter Substrate Utilization by LC/MS

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The bioavailable component of soil organic matter (SOM) is decomposed and cycled by the resident microbiome. Determining the composition of this heterogenous and poorly understood carbon pool of small molecules and metabolites is extremely challenging with the diversity of chemical classes within likely precluding global profiling using a single liquid chromatography mass spectrometry (LC/MS) approach. Here we describe our efforts to use a combination of normal and reverse phase chromatography to characterize exometabolomic extracts from a time-course culturing of a dominant denitrifying bacteria in order to monitor its substrate preference within the water soluble fraction of SOM. Aqueous extractions were done on chloroform fumigated soil samples from the ENIGMA FRC site in Oak Ridge, TN and used to supplement minimal media. SOM supplemented media was used to culture a dominant denitrifying microbe of the Rhodanobacter genus (class Gammaproteobacteria) isolated from the study site. Exometabolomics extracts were prepared from spent media fractions collected at 5 time points from mid log into stationary phase. Hydrophilic interaction (HILIC) chromatography at basic pH was used for polar metabolite separation and reverse phase (RP) chromatography at acidic pH for non-polar separation. Scan data acquisition (MS1) was done with an electrospray ionization (ESI) source operated in positive and negative mode using a quadropole time-of-flight (Q-TOF) MS followed by MS2 at multiple collision energies. Sediment extracts were found to contain a wide range of mono-, di- and oligosaccharides, amino acids, fatty acids, lipids, nucleobases, nucleotides as well as many novel metabolites. Polar and apolar separation achieved by the use of HILIC and RP chromatography increased the coverage of our application. Authentic standard curated databases with retention times specific to separation type were used to provide high confidence identifications of metabolites. Additional annotation techniques were used whereby standards data informed chromatographic regions where feature groups were annotated to metabolite class by putative molecular formula (mono, di- and oligosaccharides, lipids, fatty acids pools). MS2 fragmentation patterns substantiated the annotation of these classes of metabolites and will be a primary tool for eventual structural determination of novel metabolites. LC/MS based characterization of Rhodanobacter spp. substrate preference of bioavailable soil organic matter.

POSTER 477

Deciphering Metabolic Foodwebs in Biological Soil Crusts using Soil Exometabolomics

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Microbial metabolism and metabolite exchange in soils are not well understood. Liquid chromatography mass spectrometry (LC/MS)-based metabolomics is being used to investigate the complex foodwebs in biological soil crust (biocrust) systems. Biocrusts are communities of organisms inhabiting the upper layer of soil in arid environments. Microcoleus vaginatus, a non-diazotrophic filamentous cyanobacterium, is the key primary producer in biocrusts of the Colorado Plateau and is an early pioneer in colonizing arid environments. Over decades, biocrusts proceed through developmental stages with increasing complexity of constituent microorganisms and macroscopic properties. Since Microcoleus vaginatus does not fix nitrogen, metabolite exchange with other microorganisms presumably plays a key role in the cycling of soil organic matter and in determining biocrust community dynamics. To characterize the metabolite composition of biocrust, soil was fumigated with chloroform and extracted with water followed by analysis using LC/MS. Metabolite annotation was based on fragmentation spectra (by tandem LC/MS) and comparison with an in-house authentic standards library. Substrate preferences of individual key soil bacteria were identified by exometabolomics analysis using our LC/MS platform. Sixteen bacterial isolates were incubated in minimal media containing biocrust extracts and metabolite profiles were compared to uncultured fresh media to identify uptake and release of metabolites. Fumigation of soil prior to extraction allowed for the detection of a broad range of intracellular and extracellular biocrust metabolites including amino acids, carboxylic acids, nucleotides, sugars, sugar alcohols, and fatty acids. Exometabolite profiling revealed more uptake in general rather than release of biocrust metabolites with some degree of microbial specialization, indicating potential compatibility and competition of these isolates in the biocrust environment. The isolate data are made available through our new exometabolomics data repository, the Web of Microbes (webofmicrobes.org) and is being used to link exometabolite cycling to specific microbes within the biocrust in order to further understand nutrient exchange in soils. Novel exometabolomic profiling approaches with microbial isolates and comparison of these data are being used to assemble soil foodwebs.

POSTER 479

Microbial metabolomics and small molecule fragmental analysis and networks provide new insights in the metabolic plasticity of intestinal bacteria.

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This study shows how microbial metabolomics can provide novel insights into well-studied commensal, probiotic, and pathogenic intestinal bacteria by analysing their metabolite profiles. Metabolomics approaches offer global insight into organisms' metabolic capacities. Yet, to date, few studies have taken advantage of these methods to gain greater understanding of these important characteristics in enteric bacteria, for example, explaining different Campylobacter jejuni adaptations to availability of substrates. Thus, this study aimed to conduct detailed analysis of the small metabolite spectrum of widely-utilised Campylobacter jejuni and E. coli strains to provide the basis for further studies into the understanding of their physiology and their beneficial or damaging interactions with their hosts. Bacteria were grown under well-defined conditions in commercially available media. A generic extraction protocol was used to obtain the small polar metabolite fraction of both the supernatant of the cultures and the whole cell extracts. A column embedded with zwitterionic polymeric HILIC particles (ZIC-pHILIC) was used to separate polar metabolites and, subsequently, metabolites were analysed in a Thermo Q-Exactive in both positive and negative ionization mode. The full scan data was analysed with an in-house Glasgow Polyomics data analysis pipeline, resulting in the identification of significantly changed metabolites between treatment

and control or bacterial species. This study then used small metabolite fragmentation as foundation for metabolite annotations and molecular networking as developed by Dorrestein et al. [http://dorresteinlab.weebly.com/molecular-networking.html]. A single Campylobacter jejuni strain was grown under three conditions, namely glutamate-supplemented medium, fucose-supplemented medium, and the non-supplemented medium as control. Samples of the supernatant were taken at different time points to allow for metabolomics analysis of metabolic changes occurring over a time span of 24 hours after inoculation. Firstly, it was shown that C. jejuni uses a wider spectrum of small molecules as potential carbon source than previously thought. Moreover, multiple metabolites were depleted simultaneously rather than sequentially, indicating no single preferential carbon source. Secondly, several breakdown products of fucose could be annotated using fragmentation spectral comparisons, thereby confirming one of the postulated breakdown routes of fucose in Campylobacter jejuni. To the best of our knowledge, those breakdown metabolites were not previously annotated in bacterial extracts. Thirdly, the addition of either glutamate or fucose induced massive changes in the metabolic householding of Campylobacter jejuni, even resulting in the production of novel metabolites. An efficient mass fragmentation approach enabled the formation of informative molecular networks showing a number of treatment-specific metabolites that were not formed in the control data set. Extracts of medium supernatants of bacterial cultures and whole cells of several pathogenic E. coli strains and one probiotic E. coli strain were analysed to discover differences in their metabolite profiles that could distinguish E. coli Nissle 1917. Interestingly, the probiotic NISSLE 1917 strain produced and excreted elevated levels of the small molecule citrulline in comparison to pathogenic E. coli strains and the commensal strain E. coli MG1655. Citrulline, a key intermediate in the urea cycle, was earlier found to be a regulator of intestinal health and elevated levels were positively associated with healthy intestines. This is a novel observation and a potential mechanism of action based on small metabolite production can be postulated for this probiotic E. coli strain. Information-rich pHILIC-MS microbial metabolomics analysis resulted in novel insights in the metabolomics plasticity of Campylobacter jejuni and E. coli bacteria.

POSTER 481

Integrating Metabolomic data as constraints in flux balance models to understand antibiotic resistance

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Antibiotic resistance is a serious problem in contemporary medicine. Mechanisms of bacterial susceptibility to drugs although understood, their adaptability to such selection pressures remain unclear. The systems biology paradigm that integrates experimental data with computational models can be used to understand the emergent phenomenon of antibiotic resistance. Altered metabolism has been implicated in many diseases and has an implicit connection to antibiotic resistance. There is an urgent need to delineate this connection between uncontrolled growth and metabolism to understand and tackle antibiotic resistance. Chloramphenicol and Streptomycin both target protein biosynthesis as a part of their bactericidal action. Despite their primary action on protein synthesis, there are reports on the effect of these drugs on energy, nucleotides and amino acid metabolism. Populations of Chromobacterium violaceum (CV) ATCC 12472 were evolved in a controlled environment using two antibiotics targeting protein synthesis, chloramphenicol and streptomycin resulting in two resistant phenotypes ChlR and StrpR. The evolved populations were genotyped for genome-wide sequence changes and phenotyped for growth and antibiotic sensitivity on several Carbon/Nitrogen sources. Secondary resistance phenotypes were also obtained. A genome scale metabolic model (GSM) of CV was developed through a metabolic network reconstruction. Pathways for antibiotic metabolism were included. Targeted metabolic profiling was done using a combination of LC-MS/MS and MALDI-TOF MS. Heterogenous data obtained was integrated into the framework of the GSM. Constraint-based flux balance analysis was used to assess metabolic plasticity in the presence and absence of the antibiotics. Despite the selection pressure acting at the protein synthesis level for both the populations, differential levels of the secondary metabolite violacein were observed. Metabolic profiling showed not only differential violacein pathway intermediates but also changes in central metabolism specifically amino acid metabolism. There was a

marked increase (upto 40%) in the total violacein in the StrpR population. However, only prodeoxyviolacein was detected in the ChlR population. Dynamic profiling for 24 hours showed higher Violacein to deoxyviolacein ratio in StrpR compared to ChlR strain. Intracellular levels of proline were higher in ChlR while StrpR showed high levels of Leucine/Iso-Leucine and valine. Mutations detected using NGS were in 6 genes per evolved population and predominantly in the multidrug efflux pump repressor (acrR) and in a ribosomal protein (rpsL). The model was able to predict perturbations in growth and metabolism in the resistant phenotypes. Secondary resistance to beta lactam antibiotics was also observed. The experimental data can be used as constraints in flux balance models to explore the solution space of feasible function of a cell. The metabolic network reconstruction contained 890 metabolic genes, 1035 metabolites and 1185 reactions. A Biomass composition was determined based on literature and genome composition data and the network translated into a model amenable to mathematical analysis. The model was able to simulate growth of chromobacterium and able to predict respiration on C/N sources with high accuracy when compared to legacy Biolog (TM) data. The model was able to predict the differential violacein phenotypes of the resistant populations. The flux variability analysis results helped explore the differential metabolic reactome or operational space of susceptible and resistant chromobacterium violaceum and the altered metabolism at a systems level. Potential for understanding implication of metabolism in antibiotic resistance using genome scale flux balance model in a systems biology approach

POSTER 483

Cryptic gut bacterial metabolites that regulate colorectal cancer formation

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Members of the human microbiota are increasingly being correlated to human diseases, but the majority of the responsible microbial metabolites that regulate host-microbe interactions remain largely unexplored. Select strains of E. coli present in the human colon have been linked to initiating inflammation-induced colorectal cancer through an unknown small molecule-mediated process. The responsible nonribosomal peptide-polyketide hybrid pathway encodes “colibactin,” a largely uncharacterized family of small molecules. Genotoxic small molecules from this pathway capable of initiating cancer formation have remained elusive. Guided by comparative metabolomic analyses, we employed a combination of bioinformatics-guided isotopic labeling studies and NMR spectroscopy to characterize the colibactin warhead, an unprecedented substituted spirobicyclic structure that crosslinks DNA. The work provides direct experimental evidence for colibactin’s DNA-damaging activity. A colibactin “pathway-targeted” molecular network was constructed to assess microbial secondary metabolite biosynthesis at a systems level, to probe the effects of various experimental perturbations, and to aid downstream metabolite discovery efforts. High-resolution mass spectrometry (HR-MS)-based comparative metabolomics among wildtype and mutant organisms were carried out on an Agilent iFunnel 6550 quadrupole time-of-flight (Q-TOF) MS instrument. Comparative data analysis was conducted using the Agilent Mass Profiler Professional chemometric platform to determine unique molecular features that were dependent on a functional pathway. Unique pathway-dependent metabolites were then targeted in tandem MS (MS/MS) experiments for subsequent pathway-dependent network clustering through the Global Natural Products Social Molecular Networking (GnPS) platform. The established network was significantly enhanced through additional genetic and isotopic labeling studies. Through comparative metabolomics analyses among wildtype and mutant organisms, we constructed a pathway-dependent molecular network to characterize the colibactin pathway. Based on bioinformatics predictions of amino acid substrate utilization, we employed [U-13C]-isotopic labeling studies in various auxotrophic strain backgrounds to support a system-wide analysis of colibactin biosynthesis. The data collectively supported the structures of unstable advanced precolibactins and an unexpected biosynthetic model that accounted for at least 32 predicted colibactin pathway-dependent structures. The network highly focused downstream NMR-based structural characterization efforts of novel molecules. While it had been speculated that the unknown molecules may directly induce DNA double-strand breaks, here we demonstrate that the newly characterized colibactin warhead crosslinks DNA in vitro, supporting a new model for colibactin’s mode of action. The data supported unexpected models for both colibactin biosynthesis and its mode of action linked to colorectal cancer formation.

POSTER 484

Identification of metabolic switches in global reprogramming of metabolic flux

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Cellular growth and response to nutrients require an adjustment of the metabolic program. During this shift, metabolic enzymes undergo dramatic transcriptional reprogramming enabling them to carry metabolic flux according to condition-specific metabolic needs. GCR1 is a transcription factor in yeast that globally regulates metabolism. Despite almost 1000 key metabolic genes enzymes under the control of GCR1 have been identified, it is not clear which metabolic switches control and concert nutritional state and cellular growth. Using computational and experimental systems biology we identify metabolic switches that are conserved at the transcriptomic, metabolomics, and fluxomic level. Gene to protein to reaction rules from any multi-omics data set are mapped onto a model building algorithm and subject to flux variability analysis. Identified metabolic switches are tested by NMR analysis for determination of carbon-resolved stable isotope incorporation. Our findings show that GCR1 promotes cellular growth by rebalancing carbon metabolism. Glycolysis, one-carbon and amino acid metabolism are statistically significantly perturbed pathways at the transcriptional and flux level. GCR1 knock out shows reliance on import of alternative carbon sources instead of biosynthesis pathways. This systems biology study fuses gene expression and metabolic flux analysis generating a comprehensive picture of metabolic rewiring upon proliferation.

POSTER 485

GC-MS based profiling of central carbon metabolism in Cyanobacteria

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Cyanobacteria are photosynthetic prokaryotes that evolved about 2.5 billion years ago. They shaped the global atmosphere by decreasing the carbon dioxide (CO_2) concentration while increasing the proportion of molecular oxygen (O_2). Ribulose-1,5-bisphosphate carboxylase/ oxygenase (RubisCO) is key to the evolution of photosynthesis and catalyzes the central reaction of Ci-fixation where ribulose-1,5-bisphosphate (RuBP) reacts with CO_2 to produce two molecules of 3-phosphoglycerate (3PGA). But RubisCO also accepts O_2 as a substrate. The oxygenase reaction competes with Ci-fixation and produces equimolar amounts of 3PGA and toxic 2-phosphoglycolate (2PG). Cyanobacteria adapted to increasing atmospheric O_2 both, by largely avoiding 2PG production via the evolution of an efficient CO_2 concentrating mechanism (CCM) and by mechanisms for 2PG degradation through photorespiratory 2PG metabolism. We performed GC-MS based profiling of primary metabolism of the cyanobacterial model organism Synechocystis sp. PCC 6803 (Synechocystis) wild type shifted from high to low inorganic carbon (Ci)-availability in comparison to mutants that are defective for 2PG metabolism, Ci-regulation, and the Ci-uptake and carboxysome components of the CCM. Metabolic profiling was complemented by transcript profiling and feeding experiments with 13C-labelled substrates. When shifted from high to low Ci-availability, Synechocystis wild type exhibits characteristic changes in central carbon metabolism before full adaptation to low Ci-availability is observed, i.e. before a functional CCM is set up. This includes a transient increase in photorespiratory metabolites that we defined as the "photorespiratory burst". When we examined a mutant lacking 4 of 5 known Ci-uptake systems in Synechocystis under our experimental conditions, we expected the mutant to exhibit a strong photorespiratory burst, as seen in a carboxysome-less mutant. Instead, we observed a complex physiological plasticity of Synechocystis which compensates extreme intracellular Ci-limitation and still successfully suppresses the RubisCO oxygenation reaction. Since cyanobacteria are the evolutionary ancestors of all eukaryotic chloroplasts, our findings on photorespiration and its regulation in Synechocystis may serve as a model towards a deeper understanding of plant photorespiration. Multiple layers of Ci-regulation exist that protect Cyanobacteria of the damaging effects of Ci-limitation and photorespiration.

POSTER 486

Stable isotope-assisted analysis for cyanobacterial TCA cycle by integrating multiple analytical systems

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In order to reduce the CO2 emission, studies on bioprocesses of producing useful substances or liquid fuels using cyanobacteria, and metabolic modification strategies to achieve higher production have been widely performed. Both rapid proliferation and high productivity of the target product are required for the bioprocess using microrganisms. In order to meet these requirements, understanding the metabolic regulation mechanism of cyanobacteria is essential. Nevertheless, many important cyanobacterial metabolic pathways and their functions are still waiting to be unraveled even for primary pathways such as TCA cycle. Stable isotope tracing is known as a powerful technique for determining the fate of individual metabolites and elucidating metabolic pathways. Therefore, we focused on isotope labeling experiments for investigating cyanobacterial metabolism. Synechococcus elongatus PCC7942, a species of typical freshwater blue-green algae, was used in this study. The labeling experiment was performed by 13C-labeled acetate in cyanobacteria under light and aerobic conditions. Each isotope labeling ratio of the intermediates of TCA cycle and the related amino acids was obtained from triple quadrupole mass spectrometer. To monitor the isotopomer ratios of all targeted metabolites, we used LC-MS/MS with/without derivertization and GC-MS/MS. LC analysis was performed with ion-pair (IP) reagents before and after the sample derivertization by phenylhydrazine, and GC analysis was performed after sample derivatization by MTBSTFA. The naturally occurring isotopic effect was corrected by solving the non negative least squares problem to integrate the data from LC-MS/MS and GC-MS/MS. Although there have been a few reports focusing on the metabolism of cyanobacterial TCA cycle, several TCA cycle intermediates were not included possibly due to the limitation of current analytical methods. So far, no study investigating aldehydes, organic acids and amino acids involved in TCA cycle at the same time has been reported. Here, we constructed a stable isotope tracing method for analyzing important intermadiates of cyanobacterial TCA cycle covering the above three groups by the combination of different analytical systems. Phenylhydrazine derivertizaion enables the detection of glyoxylate, oxalacetate and succinic semialdehyde with high sensitivity, IPOSTER LC/MS method covers most organic acids and GC/MS can analyze several organic acids as well as most related amino acids. In order to integrate the isotopomer data from different analytical systems, correction of the naturally occurring isotopic effect is necessary. Therefore, we applied the non negative least square regressions to correct the data matrix of isotopomer ratios and successfully reduced the basic mass differences among analytical systems. Then, we applied the established method to a widely used cyanobacterium model, Synechococcus elongatus PCC7942. 13C-labelled acetate was used to monitor TCA cycle activity under light and aerobic conditions. As a result, acetate was found to be incorporated into the cells immediately, converted to acetyl-CoA, and subsequently entered TCA cycle. The alpha ketoglutarate (AKG) and glutamate were rapidly labeled by 13C from acetate, but the following intermediates like succinate and malate showed completely different patterns. These observations indicated that the important step of cyanobacterial TCA cycle from AKG to succinate as well as glyoxylate shunt was not active under light and aerobic conditions, which is consisted with previous studies. The method established in this study may contribute to the further understanding of functions of cyanobacterial TCA cycle by applying to different species of cyanobacteria under various growth conditions. A stable isotope tracing method for monitoring the complete cyanobacterial TCA cycle by combining different analytical systems was established.

POSTER 488

Metabolic and Morphological Changes of Chlamydomonas reinhardtii under Nitrogen-deficient Conditions using turbidstat synchronous culture system.

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Microalgal triacylglycerol (TAG) is expected for a next-generation biofuel feedstock, and a green alga Chlamydomonas reinhardtii has become a model for understanding metabolic regulation mechanisms of TAG-accumulation. Most of oil-rich green algae including C. reinhardtii accumulate starch and TAG under nutrient deficient or other stress conditions, but they rapidly grow with minimal storage materials under normal experimental conditions. C. reinhardtii had analyzed mass spectrometry-based metabolomics under nitrogen-deficient and mixotrophic conditions. Using turbidstat synchronous culture system, C. reinhardtii CC-503 were autotrophically grown in flat-flasks containing 500 mL TP medium with 1% CO2 aeration. Temperature of water jacket was 25.0 °C and light was provided in a 14/10 h day/night cycle at an intensity of 100 µmol m-2 s-1. Set point of turbidostat was optical density 0.25 at 630 nm. Logarithmic growth cells in TP replaced to TPOSTER N and collected after 1 and 2 days cultivation. Metabolome (mainly ionic metabolites and glycerolipids) were analyzed by using capillary electrophoresis- and liquid chromatography-mass spectrometry. Light and fluorescence microscopy were performed to observe morphological changes. Most of mono- and di-saccharide and TAGs were significantly increased under −N conditions. These are positively rerated to observation of starch granule and oil droplet under −N. In contrast, many of galactolipids were decreased under −N. Phospholipids containing more than 4 total double bond in acyls mainly decreased under −N, but it containing less than one mainly increased. This result may reflect the existence of two deferent metabolic flows. While proline, asparagine, lysine and arginine were decreased under −N, 9 proteogenic amino acids were increased. Most of purines and pyrimidines were decreased under −N. Under −N, average cell volume was enlarged 1.4 folds. However C. reinhardtii could not intake nitrogen source under -N, 9 amino acids were increased and cell volume was enlarged.

POSTER 489

Optimizing Metabolomics for Practical Application in Synthetic Biology

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Successful metabolic engineering of micro-organisms for commercially viable production of biochemicals requires striking a precarious balance between strain health, product flux and product export. Amyris's synthetic biology platform depends on optimizing each of these performance parameters through a standardized design-build-test-learn process. Comprehensive systems biology approaches are used to thoroughly interrogate engineered strains to inform the next iteration of strain design in a data-driven fashion. A targeted metabolomics platform underpins our ability to enhance the design of complex biosynthetic pathways. To assist in the characterization of metabolite concentrations and their fluxes, the Amyris metabolomics platform has been developed for the analysis of a wide range of microbial metabolism and can be applied to analysis in microtiter plates to commercial-scale fermentors. Automated sampling and extraction procedures focus on maintaining sample metabolic integrity through rapid sampling, instantaneous quenching, and efficient extraction. Several complementary platforms including LC and LC-MS/MS, GC and GC-MS allow for the quantitation of up to 90 distinct metabolites representing central carbon metabolism, product pathways, intermediates, byproducts and membrane components. Data quality is maintained through the use of C13 Internal standards for each analyte of interest and process metrics are incorporated into each step of the platform to ensure consistent extraction and instrument performance. Sample barcoding and an integrated data pipeline serves to ensure that all sample and meta-data is captured, systematic normalization schemes are applied and all data is properly archived for retrieval via a SQL data warehouse. Finally metabolomics data is assembled on its own or with other omics data for biological contextualization in the form of pathway and lineage mapping. A summary of the metabolomics capability and its application in Amyris's synthetic biology platform are presented.

POSTER 491

Comparative Metabolomics of Toxic and Non-Toxic Microcystis Strains Unravel The Biological Function of Cyanotoxin.

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Harmful algal blooms, the accumulation of toxin-producing cyanobacteria (blue-green algae), has become an emerging global health issue in freshwater habitats worldwide. The increasing occurrence and global distribution of toxic algal blooms not only have adverse impact on the biodiversity in temperate and tropical lakes worldwide, but also compromise water quality and safety. Algal blooms in Singapore reservoirs are mainly attributed by Microcystis sp and Anabaena sp, and the main toxin producer is Microcystis sp. Interestingly, not all Microcystis sp. present in local reservoirs are toxin (microcystin) producers. In this presentation, a combination of conventional toxin determination methods, and metabolomics approaches were used to reveal why toxic and non-toxic Microcystis sp. coexist in local reservoirs. Two model Microcystis stains (PCC7806, NIVA-CYA43) and six Microcystis isolates from Singapore reservoirs were cultured under high light exposure (60 µmol photons/ m2.s) to simulate the strong daylight condition on an average clear day in Singapore. Cell toxicity was validated through quantitative polymerase chain reaction (qPRC) and enzyme-linked immunosorbent assay (ELISA), which we confirmed that 4 strains were toxigenic and the others were non-toxigenic. Cell morphology, cell density, dissolved oxygen concentration, chlorophyll a content, total carbohydrates concentration were determined in the respective cultures at early stationary phase. Subsequently, the metabolome of these toxic and non-toxic Microcystis strains were determined by NMR and LC-ESI-QTOF in positive and negative modes. A comparison of cell morphology and cell density of toxic and non-toxic Microcystis strains showed that the energy intensive toxin production process has no adverse effect on cell morphology (unicellular, spherically shaped, 2-4 um), but compromises on cell growth as the cell density of toxic strains is appromixmately 2-3 folds lower than the non-toxic strains. Under similar high light treatment, toxic cultures were found to have higher dissolved oxygen, chlorophyll-a, and total carbohydrates contents. These parameters suggest that toxic Microcystis strains could undergo photosynthesis more efficiently under high light conditon. In our comparative metabolomic study, we observed that non-toxic Microcystis strains have lower sucrose and sucrose precursor (glucose-1-phosphate, glucose-6-phosphate and UDPOSTER glucose). Since carbohydrates and oxygen are the main product of photosynthesis, substantial decreased in sucrose and dissolved oxygen content indicates that photoinhibition as a consequence of high light exposure has greater impact on non-toxic strains than toxic strains. The accumulation of several by-products from phospholipid degradation, a process to increase fatty acid concentrations for beta-oxidation, further suggests photosynthesis impairment in non-toxic strains. The accumulation of 3-hydroxybutyrate, monomers of polyhydroxybutyrate which serve as carbon and energy source in cyanobacteria, suggest that toxin in toxigenic Microcystis strains could have play an essential role in photoinhibition resistance. Furthermore, the upregulation of phosphatidylglycerol and respective changes to other metabolites in the metabolic pathway supported that photosynthetic machinery in toxic strains is more stable than in the non-toxic stains. To understand whether the metabolic responses of these toxic and non-toxic strains to high light exposure are solely influenced by cyanotoxin, a separate photoinhibition study where the cultures are exposure to extremely high light intensity (250 µmol photons/ m2.s) is ongoing. First comparative metabolomic study using NMR and LC-ESI-QTOF to unravel the biological functions of cyanotoxin in Microcystis.

POSTER 492

When less is more: Understanding non-native carbon utilization and carbon signaling molecules

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Cellulosic materials could make significant contributions to meet the rising demand for bio-based products and energy. The co-fermentation of cellobiose derived from cellulose and xylose derived from hemicellulose allows the sugars to be consumed simultaneously and may enable an economical continuous process. Despite a minimal heterologous gene requirement—cellodextrin transporter and intracellular beta-glucosidase, cellobiose utilization pathway exhibits an inferior consumption rate than that of glucose in Saccharomyces cerevisiae. In this study, we utilized metabolite-profiling approach to examine the effect of cellobiose utilization in S. cerevisiae. Engineered cells subjected to anaerobic fermentations supplied with either cellobiose or glucose as their sole carbon sources were analyzed. We hypothesize that (1) ATP deficiency and (2) low glycolytic flux will be observed in cellobiose-grown cells in comparison to glucose-grown cells. First, the heterologous cellodextrin transporter is a proton symporter. Under anaerobic condition in which ATP synthesis is limited to substrate-level phosphorylation, the energy currency will be used up for cellobiose uptake and maintenance of the heterologous protein expression. Second, the lack of extracellular glucose, which serves as a signaling molecule, may result in limited activity of glycolysis when cellobiose is provided as a sole carbon source. We applied system biology to study the effect of non-native carbon utilization and the role of carbon signaling molecules.

POSTER 493

Dynamic metabolic profiling of cyanobacteria for bio-based chemical production

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To ensure reliable sources of energy and materials, the utilization of sustainable biomass has considerable advantages over petroleum-based sources. Photosynthetic algae have attracted attention as a third-generation feedstock for production of bio-fuels and bio-based chemicals, because algae cultivation does not directly compete with agricultural resources. Cyanobacteria are a promising feedstock because of their high photosynthetic capability and glycogen content. Intracellular glycogen can be converted to commodity chemicals such as lactate and succinate by autofermetation. The aim of this study is to determine the metabolic flux of glycogen biosynthesis and catabolism in cyabanocteria using a dynamic metabolomic approach. Time-course profiling of widely targeted metabolic intermediates in the cyanobacterium Synechocystis sp. PCC6803 was performed using capillary electrophoresis coupled to time-of-flight mass spectrometry. Also, in vivo labeling with NaH13CO3 and 13C-glucose was carried out to measure the turnover of metabolic intermediates including sugar phosphates, sugar nucleotides, organic and amino acids in the cyanobacterium. The combination of in vivo 13C-labeling of metabolites and metabolomic analysis revealed that glycogen is biosynthesized with carbon derived from amino acids released from proteins via gluconeogenesis under conditions of nitrogen source-depleted concition. Under dark and anaerobic condition, carbon flow from sugar to succinate was unveiled This dynamic metabolic profiling approach provided conclusive evidence of temporal alterations in the metabolic profile in cyanobacterial cells. Elucidation of carbon flow in glycogen biosynthesis and catabolism in cyanobacteria

POSTER 494

Metabolic profile of genetically engineered Saccharomyces cerevisiae to produce friedelin

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Friedelin is a triterpene that show accumulation in leaves of Maytenus ilicifolia (Celastraceae). Friedelin is a putative precursor of the quinonemethide triterpenes, maytenin and pristimerin, exclusively found in the root of Celastraceae species and occur as minority compounds. In order to improve the amount of friedelin and quinonamethide triterpenes, a project dealing on metabolic engineering, using as a host

Saccharomyces cerevisiae, has been developed and the possibility to understand the cellular response against the metabolite produced provides the opportunity to discover mechanisms involved in such biosynthesis and increase the production of target metabolite. Therefore, this study aim to analyse the metabolomic profile of the Saccharomyces cerevisiae after transfer the gene encoding friedelin synthase, and evaluate the resulting metabolic disorder. The coding sequence of MiFRS was cloned in the expression vector pYES2. The Saccharomyces cerevisiae strain used in this work was designed increasing the precursors of ergosterol and decreasing the formation of lanosterol synthase (VZL1303). Yeast was cultivated in the synthetic complete medium without uracil and 2% of galactose was added to promove the induction of the heterologous sequence of friedelin synthase. Control was constituted by cultivating the yeast strain transformed with the vector empty. Samples of culture were submitted to quenching procedure and extracted with boiling ethanol solution (75% v/v water). After centrifugation, the supernatant was lyophilized, and derivatization reactions were performed (methoxymation and silylation). The resulting solutions were analyzed by GC-MS. A metabolome analysis is a powerful tool to understand metabolic changes in response to genetic modification. The metabolome samples of S. cerevisiae genetically modified was obtained using quenching proceedings and spectrometric analysis. The analytical technique based on GC-MS was chosen mainly to provide ease of sample preparation methodology as well as the availability of libraries for identification of such compounds. The preliminary results show that the main differences between the genetic modified strain and the control were observed in the triterpene pathway. In addition to the expected production of friedelin, was also observed the formation of other products, including b-amyrin and huge output lupeol, indicating the activity of the terpenoid biosynthetic modules as an efficient system to the synthetic production of new secondary metabolites. Moreover, the global comparison showed the overall change in the metabolism involved in other classes of compounds, mainly involved in the disturbance in the biological system. Was also possible to observe differences in the level of production of primary metabolites as sugars and amino acids, that may be associated with cellular stress. Differential metabolite data offer many avenues for analysis and interpretation, besides promising glimpse of future systematic integration of differential metabolite levels of yeasts with synthetic biology and gene expression. Contribution of metabolomic studies related to biological synthesis of friedelin, invaluable to better understand of the biosynthesis and metabolic disorder.

POSTER 495

Metabolomics-driven identification of the rate limiting steps in transgenic Escherichia coli 1-butanol production

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1-Butanol is an important chemical feedstock and gasoline substitute. Previously, 1-butanol producing transgenic E. coli strain has been constructed by introducing a modified Clostridial CoA-dependent pathway. High titer production was achieved by introducing NADH and acetyl CoA driving forces to direct the flux towards 1-butanol production. Despite some success, further strain improvement is needed to reach an industrially-competitive level of 1-butanol production. Metabolic profiling is a powerful tool for metabolic engineering because it enables us to characterize cellular phenotype with high resolution. In this study, metabolomics technique was applied to identify the rate limiting steps in 1-butanol production system. Metabolic profiling of two 1-butanol producing strains, JCL166 and JCL299 (JCL166 with pta gene deletion) was performed to visualize the effect of pta deletion in the highest producing strain. The strains were cultivated in four different media under anaerobic condition. Cells were collected by fast filtration and quenched by rapid cooling in liquid nitrogen. Metabolite extraction was performed by methanol, chloroform and water. Hydrophilic low molecular metabolites were analyzed by GC-Q/MS abd ion pair LC-QqQ/MS and multivariate analysis of metabolome data was performed by SIMCA-P. Measurements of extracellular alcohols and organic acids were performed by GC with flame ionization detector and LC with photodiode array detector. pta gene encoding phosphotransacetylase for the synthesis of acetic acid has been the target of metabolic engineering to reduce the flux to acetate and increase the production of commercially desired end products. JCL299 showed higher 1-butanol

production compared to JCL166 in all media as a result of pta gene deletion. However, significant accumulation of by-products such as pyruvate and butyrate in the media were also found. Orthogonal Projection to Latent Structure-Discriminant Analysis (OPLS-DA) also revealed the accumulation of intracellular butyryl-CoA in JCL299. These results indicated that the reduction of butyryl-CoA to 1-butanal is a rate-limiting step in the 1-butanol production and this resulted in undesired accumulation of butyrate. The result of this work demonstrated the usefulness of metabolomics technique to gain insight of CoA-dependent 1-butanol production pathway and to effectively identify the rate limiting step in 1-butanol production This is the first demonstration of utilizing metabolomics to identify rate limiting steps in E. coli 1-butanol production

POSTER 496

New insights on the 1-butanol-producing pathway in transgenic cyanobacteria using quantitative target analysis and kinetic profiling

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Cyanobacteria have been long considered as essential organisms for achieving a low carbon society as they are able to convert atmospheric carbon dioxide into various industrial products. The CoA-dependent pathway based on Clostridial acetone-butanol-ethanol (ABE) fermentation has been successfully engineered and modified in the cyanobacterium Synechococcus elongatus PCC7942 to produce 1-butanol. However, efforts to improve 1-butanol production from this strain using classical metabolic engineering techniques have proven to be a challenge. Therefore, this study aims to use quantitative target analysis and kinetic profiling of intracellular intermediates in the CoA-dependent pathways to gain insights for further CoA-dependent pathway optimization for the improvement of 1-butanol productivity in S. elongatus. Targeted metabolic profiling of four different strains of cyanobacteria (wild-type PC7942 and three 1-butanol producing cyanobacteria EL14, EL 22, BUOH-SE) was performed. Absolute concentration of key intermediates in clostridial 1-butanol pathway, namely acetyl-CoA, malonyl-CoA, butyryl-CoA and pyruvate was quantified using 13C-labeled cell extract as internal standard. Cells were harvested by fast filtration, washed with 70 mM ammonium bicarbonate, and quenched with liquid nitrogen. Metabolite extraction was performed using 80% methanol, chloroform, and water. Metabolic turnover analysis was conducted using NaH13CO3 labelling experiment. Metabolite measurement was performed using a reversed phase ion-pair liquid chromatography-triple quadrupole mass spectrometry (RPOSTER lPOSTER LC/QqQ-MS) analysis with multiple reaction monitoring (MRM). Quantitative target analysis indicated that the rate-limiting steps in the cyanobacterial 1-butanol biosynthesis were the conversion of pyruvate to acetyl-CoA and the reduction of butanoyl-CoA to butanal. In addition, introduction of the pduP gene in the BuOH-SE strain unexpectedly increased acetyl-CoA synthesis. Furthermore, a high intracellular pyruvate to acetyl-CoA concentration ratio was found in the strain that is unable to regenerate free CoA from butanoyl-CoA (EL9+pEL256) indicating that regeneration of free CoA from butanoyl-CoA is important for the conversion of pyruvate to acetyl-CoA. Quantitative target analysis and kinetic profiling found new insights in the CoA-dependent pathway are useful for future metabolic engineering designs.

POSTER 497

The combined use of metabolome data from rat plasma and liver cell system for determination of toxicological equivalency of enantiomers

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BASF and metanomics have developed the data base MetaMap®Tox containing toxicity- and metabolome data for more than 700 reference compounds derived from 28 day rat studies. More than 120 toxicity-specific rat plasma metabolome patterns were identified. Recently it was shown that toxicity-specific metabolic changes can also be detected on cell-based metabolome data gained from an in-vitro approach. Chiral properties of molecules may result in different biological effects. In such cases it might be recommended to only synthesize and market the biologically active enantiomer. To prove the toxicological equivalency between racemates and their corresponding pure enantiomers extensive toxicological “bridging studies” are required. Here, we report that metabolomics investigation can be used to show toxicological similarity of enantiomers and their respective racemates. The rat plasma metabolome of two agrochemical compounds (racemic and pure enantiomer) after 28 day was evaluated in terms of number and extent of metabolite changes, comparison of the metabolite changes against the patterns in the MetaMap®Tox data base as well as statistical correlation analyses of the rat plasma metabolome profiles. Cell based systems become consistently more important as serious alternative to animal testing. As a consequence BASF and metanomics developed the metabolome analysis of HepG2 cells to support toxicity assessment (in-vitro metabolomics). No relevant differences in the rat plasma metabolome profiles between the two forms of the respective active ingredients were observed. Using total rat plasma profile comparison, the best correlation for the metabolite profile of the racemic mixtures was always their active isomer showing. As the toxicity of the tested racemates and the biological active forms were known to be equivalent, this study is a proof of concept for equivalency testing. The in-vitro metabolome data gained from dosing the two above mentioned agrochemicals to the HepG2 cell system additionally confirm the results derived from the rat-plasma metabolome. Combination of metabolome data derived from in-vitro and in-vivo approach raise predictability of toxicological effects to higher level of evidence.

POSTER 498

An NMR fingerprinting based antimalarial drug screening for novel modes of action.

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Malaria is a mosquito borne tropical disease and one of the biggest killers in the world today. Multiple drugs are available for treatment of malaria, however increasing number of resistance cases even to combination therapies demand for alternative treatments. Over 20,000 of compounds have been found to have antimalarial effect in recent "black-box" screenings. Due to overwhelming number of leads high throughput methods are needed for further selection of compounds. We hypothesize that a drug with novel mode of action would be less likely to lead to resistance. In this study we develop a metabolomic fingerprinting method based in nuclear magnetic resonance (NMR) spectroscopy for rapid screening of antimalarial compounds for novel modes of action. We grow synchronous P. falciparum cultures in standard conditions in tightly controlled metabolic environment. Prior to drug exposure the cultures are pooled in order to diminish batch effect. For sample metabolite extractions we have developed a method using acetonitrile, methanol and water. For NMR data acquisition we use a 800MHz Bruker spectrometer with a Carr-Purcell-Meiboom-Gill (CPMG) pulsetrain. It allows for rapid acquisition of high quality 1D hydrogen spectra and avoids residual protein signal. For data processing and analysis we employ our custom software implemeted in Python programming language as well as R statistical software. We have developed a method for efficient metabolite extraction from P. falciparum infected human red blood cells (RBCs) using acetonitrile, methanol and water which proved to be superior to similar techniques found in literature. Furthermore it does not require parasite removal from the RBCs therefore is applicable over the whole intraerythrocitic life cycle. We have shown that our method could differentiate between P. falciparum infected and uninfected RBCs as well as various life cycle stages of the parasite. However in order to investigate finer perturbations in the parasite metabolome due to drug action in the background of RBC metabolites a more robust method was required. Due to standard data processing in NMR being rather manual and arbitrary, we needed a more consistent method that would require minimal human intervention. To address this problem we have developed a custom software for fully automated processing of NMR data. It has been implemented in Python programming language and is used for high

throughput NMR data import, processing, quality control and analysis. We have designed and performed pilot time-course drug exposure experiments the results of which will be presented. P. falciparum metabolite extraction procedure for NMR spectroscopy. A high throughput NMR data processing and analysis pipeline.

poster 500

LC-MS metabolomic response of drug-treated tumor cell lines as screening model for cytotoxic marine compounds.

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The isolation of lead compounds in natural products has become increasingly difficult, expensive and time-consuming. In order to improve the discovery of novel drugs, new methodologies capable of providing information about the processes and interactions involved in the pharmacological responses of bioactive matrices has been proposed. One of the promising tools in a systemic approach is the use of metabolomics. Metabolomics is the study of global metabolite profiles in a biological system under a given set of conditions. This approach can offer an integrated view of the biochemistry of a complex mixture and the biological variations in a specific organism. In cancer research, metabolomics has been applied for biomarkers detection and metabolic differentiation between normal and tumor cell lines. In this work we propose the development of a LC-MS based method for screening the global metabolic changes induced by drug interventions in primary cells and cell lines. Additionally, as a large screening application, we plan to profile the metabolomic response of cancer cells to a library of marine natural products. The metabolic analyses involved the use of mass spectrometry (MS) coupled with different chromatographic methods, chemometric tools for data interpretation. The method allow the analysis of tumor cell lines exposed to anticancer drugs belonging to different well-described cytotoxic mechanisms in order to provide an objective classifier for potential anticancer drugs. After determination of the metabolic profile based on well-characterized targets, the LC-MS data should be hierarchical clustering in order to distinguish the modes of action and verify the response similarity among molecules with a similar cytotoxic mechanism. According to the establishment of potential metabolomic screening method, a set of cytotoxic marine natural products with unknown mechanisms of action will be analyzed. With the results, a potential metabolomic screen method should be avaliable with applications in cancer drug development.

poster 501

Phenotyping Drug Polypharmacology via Eicosanoid Profiling of Human Whole Blood

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It is widely accepted that small molecule drugs, despite their selectivity at primary targets, exert pharmacological effects (and safety liabilities) through multiple pathways. Profiling of metabolites produced as part of physiological responses to pharmacological stimuli provides a unique opportunity to explore drug pharmacology. Here we describe a molecular phenotyping study of human whole blood, by measuring eicosanoids derived from the conversion of membrane phospholipids to arachidonic acid, an early and critical event in the initiation and establishment of multiple inflammatory responses. Treating human whole blood with various compounds at the same target protein allows us to observe unique pharmacological responses of an individual with unprecedented resolution, including both on-target and off-target effects. Human whole blood samples were stimulated with calcium ionophore or lipopolysaccharides, with or without pre-treatment with clinical and experimental molecules targeting prostaglandin and leukotriene biosynthesis, followed by 96-well plate solid phase extraction. Extracted lipids were subjected to high-throughput quantitative UPLC-MS analysis in scheduled MRM mode. A special software was developed in-house for raw data processing and quantification. Quality control using

principal component analysis for outlier sample detection, differential analysis of eicosanoids in response to biological perturbations, and hierarchical clustering were conducted using ArrayStudio. To predict in vivo pharmacology using mouse endotoxemia model, mice were pre-dosed with compounds followed by intraperitoneal administration of LPS. Blood samples were drawn after animals were sacrificed. TNFα were then quantified employing ELISA kits. 122 eicosanoid lipids in human whole blood were measured from 10 different healthy donors upon stimulation with several inducers of immunological responses and treatment with small molecule modulators of prostaglandin and leukotriene biosynthesis. Such analysis revealed differentiation between drugs nominally targeting different eicosanoid biosynthetic enzymes, and even those designed to target the same enzyme. Profiled pharmaceutical agents, including clinical and investigational molecules, affect eicosanoid biosynthesis in ways that cannot be predicted from information on their intended targets. As an example, we employed this platform to discriminate drugs based on their ability to silence prostaglandin biosynthesis in response to bacterial lipopolysaccharide, demonstrating differential pharmacological activities in an in vivo model of endotoxemia. Some of the observed effects are subject to variability among individuals, indicating a potential application of this methodology to prescreen patients entering clinical studies via a simple blood ex vivo test. Two compelling lessons emerge from our analysis. (1) Lipidome-focused profiling provides a unique scope for observing the biochemical mechanisms of action of experimental and physiological inducers of inflammatory responses and anti-inflammatory mechanisms impinging directly or indirectly on arachidonic pathway. This is not surprising, as the metabolome is the spoken language of proteins as they interact with each other directly through physical contact, or indirectly through modulatory effects mediated by biosynthetic intermediates and secondary messengers. (2) Even for the best characterized physiological agents the pharmacological responses are complex. Thus, traditional approaches that rely on target proximal or distal signaling events to assess dose-effect relationships (target biology) are incapable in displaying the true (poly) pharmacology of essentially all therapeutics. Our eicosanoid platform described here exemplifies a sophisticated tool to fingerprint target pharmacology in broad scope and fine detail, with a throughput to handle hundreds of samples at a time. Improved eicosanoids platform applied to human whole blood ex vivo enables sensitive detection of small molecule drugs' polypharmacology.

poster 502

Identification of Putative Metabolites of Norcoclaurine and Coclaurine in Human and Rat Hepatoma Cells Using Untargeted Global Metabolomics

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Norcoclaurine (aka higenamine) is a tetrahydroisoquinoline phytochemical that is found in some products marketed as dietary supplements. There is only limited data on the in vivo metabolism of norcoclaurine, but a presumptive metabolic pathway is through methylation by the enzyme catechol-O-methyltransferase (COMT) to coclaurine. This is reported to be a detoxification reaction. As a preliminary to conducting in vivo safety studies on higenamine, the metabolism and metabolomics profiles of both higenamine and coclaurine were evaluated in vitro using metabolically competent human (HuH-7) and rat (MH1C1) liver cell lines. Cells were treated with norcoclaurine or coclaurine at 100 µg/mL for 48 hours in 24-well culture plates. An aliquot of culture medium was deproteinized with acetonitrile, then 3 µL sample was analyzed by LC/MS on an Agilent Zorbax HILIC Plus column and Agilent 6520B QTOF. Data were collected in both positive and negative ion mode. Molecular features were identified from total ion chromatograms using Agilent Mass Hunter software. Initial metabolite identification focused on compounds present only in the treated group. Putative metabolites were then further identified using lists of know mass changes associated with phase I and phase II liver metabolism. For metabolomics, the Agilent Personal Metlin database was used for compound identification by matching exact mass (± 10 ppm). Untargeted global metabolomics examination of the supernatant culture medium, which is regarded as an in vitro surrogate for blood plasma, identified twelve putative metabolites of norcoclaurine. About 60% of the norcoclaurine was metabolized by the HuH-7 cells. The major metabolite, accounting for over 90% of the identified metabolites, was coclaurine confirming COMT as the major metabolic pathway for norcoclaurine. In addition to coclaurine, eleven additional metabolites were putatively identified. Additional reactions included desaturation, hydroxylation, and both sulfate and glucuronide conjugation. A similar series of

metabolizing reactions was observed for coclaurine regardless of whether it was the primary substrate or derived from enzymatic methylation of norcoclaurine. Significant (p 1.5) metabolomic changes in the supernatant medium from either norcoclaurine or coclaurine treatment included increased utilization of glucose (↑5-fold) and glutamine (↑3-fold) suggesting increased flux through the TCA cycle. Similarly, levels of markers of oxidative stress such as methionine sulfoxide (↑2.5-fold) were increased in the medium following treatment with either compound. Finally, markers of mitochondrial function such as 3-methylglutaconic acid (↓2.6-fold) and hydroxymethyglutaric acid (↓2.6-fold) were decreased in the medium following treatment with either compound. Metabolomic profiles were similar in the MH1C1 cells. These findings suggest that norcoclaurine is extensively metabolized by both human and rat liver cells and that norcoclaurine and/or its metabolites can stimulate flux through energy utilization pathways, decrease mitochondrial function, and increase generation of reactive oxygen species. Further study is needed to determine whether these metabolic changes are pathologic or adaptive and to assess the relevance of the tested concentration to real life exposures. HuH-7 and MH1C1 cells are useful for predicting both metabolites and species differences in metabolism of xenobiotics present in foods.

poster 503

Oxylipid profile of low-dose aspirin exposure- A pharmacometabolomics study

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The oxidation products of polyunsaturated fatty acids (PUFAs), or oxylipids, are potent mediators of both pro- and anti-inflammatory processes. Oxylipids can be formed by COX and represent a logical set of metabolites that could impact variability in aspirin response.The oxidation products of polyunsaturated fatty acids (PUFAs), or oxylipids, are potent mediators of both pro- and anti-inflammatory processes. Oxylipids can be formed by COX and represent a logical set of metabolites that could impact variability in aspirin response. Metabolomics allows for extensive measurement of small molecules in biological samples, enabling detailed mapping of pathways involved in drug response. We used a mass-spectrometry-based metabolomics platform to investigate the changes in the serum oxylipid metabolome induced by an aspirin intervention (14 days, 81 mg/day) in healthy subjects (n=156) who were participants in the Heredity and Phenotype Intervention (HAPI) Heart Study. Participants were chosen for oxylipid measurement based on sex-specific quartiles of aspirin response measured by post-aspirin collagen-stimulated platelet aggregation (non-COX1). Metabolic profiling of 30 oxylipids was conducted pre- and post-aspirin exposure using LC-MS and AA and linoleic acid (LA) were measured using GC-MS. We observed a global decrease in serum oxylipids in response to aspirin (26 metabolites decreased out of 30 measured) regardless of sex and independently of their fatty acid precursor or synthesizing enzyme. This decrease was concomitant with a significant decrease in serum linoleic acid levels (−19%, p=1.3 10-5), one of the main precursors for oxylipid synthesis. Interestingly, several linoleic acid-derived oxylipids were not significantly associated with arachidonic-induced ex vivo platelet aggregation, a widely accepted marker of aspirin response, but were significantly correlated with platelet reactivity in response to collagen. We hypothesized that aspirin might be disrupting the metabolic pathway somewhere upstream of these oxylipids (e.g. fatty acid precursors). To test this hypothesis, we measured levels of free AA and LA. Free AA levels were not changed by aspirin treatment (-8%; p=0.42). Free LA levels were significantly decreased (-19%; p=1.2x10-5). LA-derived oxylipids were significantly correlated with LA whereas no

correlation between AA-derived oxylipids and AA was found. We also learned that low dose aspirin broadly decreases circulating fatty acid levels in healthy adults. Pharmacometabolomics allowed for more comprehensive interrogation of mechanisms of action of low dose aspirin and of variation in aspirin response. Our results suggest that linoleic acid-derived oxylipids may contribute to the non-COX1 mediated variability in response to aspirin.

poster 504

Pharmacometabolomics of Estradiol and Progesterone Treatment in Women with Premenstrual Dysphoric Disorder

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Premenstrual Dysphoric Disorder (PMDD) affects 2-8% of women and is characterized by distressing affective and behavioral symptoms during the luteal phase of the menstrual cycle and remits within a few days after menses onset. Despite the linkage of symptoms to the luteal phase of the menstrual cycle, no abnormality of ovarian steroids has been consistently identified in PMDD compared with asymptomatic women. Nonetheless, we previously demonstrated that physiologic doses of estradiol(E) or progesterone(P) cause a recurrence of PMDD symptoms in women whose PMDD was in remission after GnRH agonist (Lupron)-induced ovarian suppression. Here, we use a pharmacometabolomics approach to examine steroid metabolites in women with PMDD and controls during GnRH agonist–induced ovarian suppression sequentially treated with E and P. We tested 49 metabolites covering the steroidogenesis pathway in 15 controls and 15 women with prospectively confirmed PMDD. All women received Lupron (3.75 mg IM daily) for 6 months, after 3 months of Lupron, all women were randomly assigned to replacement therapy of either transdermal E(0.1 mg/day) or transvaginal P(200 mg BID) for 4 weeks. After a 2-week washout, a crossover was performed. We tested significant effects on metabolite levels for the 3 hormone conditions using non-parametric Wilcoxon rank-sum tests and corrected for multiple comparisons using a false-discovery rate approach ($q<0.25$). Subsequently, the steroidogenesis pathway was separated into 21 distinct arms based on existing steroid metabolic pathways (KEGG), and each arm was tested for enrichment using a correlated Lancaster procedure. Ovarian steroid replacement of either E or P resulted in significant changes in steroid metabolites for both control and PMDD subjects. In all women (PMDD and controls) treated with P, we observed significant increases in pregnenolone, 20a-hydroxy-5a-pregnane-3-one, 20a-dihydroprogestrone. In addition, after P, significant increases in 17-hydroxyprogesterone and 17a,20a-dihydroxyprogesterone were observed in controls but not PMDD women. In all women treated with E, significant increases in estrone-SO4 were observed, and significant increases in Estradiol-3-SO4 were observed in control women only ($q<0.05$). No diagnostic differences were observed in pathways related to the ring A-reduced neurosteroid metabolites of P. Finally, significant differences in the steroidogenesis pathway arm containing estrone, E, and estradiol-17b-sulfate were detected for controls (adjusted $p=9.51x10-05$) but not PMDD (adjusted $p=0.11$). Collectively, these results point to potential differences in E and P metabolism in women with PMDD and may provide new insight into the underlying etiology of PMDD. Abnormal metabolic disposition of E and P might underlie symptom expression in PMDD.

poster 506

Pharmacometabolomics Elucidates Mechanism of Statin-Induced Insulin Resistance and New-Onset Type II Diabetes Mellitus

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Statins are the most widely prescribed drugs for treatment of cardiovascular disease, reducing low density lipoprotein cholesterol (LDL-C) levels by inhibiting the rate-limiting step in their biosynthesis. Although statins provide major benefits in reducing atherosclerosis, heart attack and stroke, they are associated with a variety of serious adverse effects that may result in premature discontinuation of treatment, including new onset type II diabetes mellitus. The objective of this proposed work was to identify changes in intermediary metabolism associated with statin-induced increased plasma glucose and biochemical processes associated with type II diabetes mellitus (T2DM). Metabolomic analyses of human plasma pre and post statin treatment from 100 participants of the Cholesterol and Pharmacogenetics (CAP) study were randomly selected from the 944 total participants. Healthy, drug-naïve patients were treated with 40 mg/day simvastatin for six weeks and LDL-C, plasma glucose, insulin, HOMA and metabolomics by gas chromatography-time of flight mass spectrometry (GC-TOF MS) were evaluated. Metabolomics by GC-TOF MS + derivatization provides valuable insight into the biochemical status of a system, including levels of amino acids, keto-acids, sugars, fatty acids and other primary metabolites that play critical roles in blood glucose regulation. We have used this approach to elucidate the differences in metabolic signatures associated with increased blood glucose and development of insulin resistance. We found that plasma glucose, insulin and insulin resistance measures were correlated with several amino acids directly linked to glucose metabolism, including glutamate, alanine and glycine, as well as several fatty acids that could feed into gluconeogenesis. Within this short six week study, some patients developed overt diabetes, while several others became hyperglycemic and developed insulin resistance. We found that simvastatin treatment induced insulin resistance and altered insulin secretion in susceptible individuals. Pharmacometabolomics identified several primary metabolites associated with increased T2DM, thus establishing a metabolic signature for statin-induced adverse effects.

poster 507

A new ligand for PPAR gamma, CB01, induces glucose transport via PPAR gamma-adiponectin-AMPK pathway and ameliorates dexamethasone-induced insulin resistance.

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Insulin resistance in a common feature of obesity and predisposes the affected individuals to a variety of pathologies, including type 2 diabetes and cardiovascular diseases. Peroxisome proliferator-activated receptor gamma (PPARr) is a member of the nuclear receptor superfamily of ligand-activated transcription factors. It is highly expressed in adipose tissue and regulates diverse biological functions, including adipocyte differentiation, lipid and glucose metabolism, and inflammation. Adiponectin is an anti-diabetic and anti-atherogenic adipokine. Plasma adiponectin levels are decreased in obesity, insulin resistance and type 2 diabetes. Adiponectin receptor 1(AdipoR1) and Adiponectin receptor 2(AdipoR2) are predicted to contain seven-transmembrane domains. AdipoR1 and AdipoR2 serve as the major receptors for adiponectin in vivo, with AdipoR1 activating the AMPK pathways and AdipoR2 activating the PPAR pathways. CB01 were evaluated for its ability to restore impaired glucose uptake and, expression of molecular markers in the adipogenesis pathway dexamethasone-induced in 3T3-L1 adipocytes using 2-[3H] deoxy-glucose uptake assay and RT-PCR. In this study, we investigated whether CB01-a piperazine derivates improves insulin resistance via PPARg-mediated in 3T3-L1 adipocytes. To explore the potential effects of the CB01 on adipogenesis, 3T3-L1 preadipocytes were differentiated with the derevatives for 8 days in combination with the insulin, dexamethasone, and IBMX. Lipid droplet accumulation in adipocytes was significantly greater with the CB01 treatment than without the CB01 treatment. We also examined that CB01 enhances adipogenesis, with increased expression of gene encoding PPARg, CCAAT/enhancer binding protein(C/EBP)-a, Glucose transporter type 4 (Glut4), AdipoR1, and adiponectin. CB01 significantly increased insulin-induced 2-deoxyglucose (2-DOG) uptake

in 3T3-L1 adipocytes. Furthermore, we determined whether CB01 regulates adipocyte lipolysis. An insulin resistant model has been made by dexamethasone on 3T3-L1 adipocytes. CB01 restored the dexamethasone-inhibited the expression of specific genes involved in adipocyte differentiation and increased glucose uptake ability. Adiponectin ameliorated insulin resistance and glucose tolerance via multiple mechanisms including activation of AMPK, decreased oxidative stress, decresed tissue triglyceride content and suppression of inflammation. We examined whether CB01 could activate AdipoR1 and AdipoR2 pathways in the adipocytes. The treatment of 3T3-L1 adipocytes with CB01 caused an increase in the phosphorylation of Thr 172 in the a-subunit of AMPK (aAMPK). Our results suggest that CB01 effectively restored dexamethasone-induced insulin desensitization via PPARg-adiponectin-AdipoR1-mediated AMPKa phosphorylation. We suggest that CB01 is novel PPARg agonists and is potentially useful for treating type 2 diabetes.

poster 508

Application of pharmacometabolomics to predict busulfan pharmacokinetics in pediatric patients

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Pharmacometabolomics is an global biochemical approach that can transform our understanding of mechanisms of drug action and variation in drug response such as drug efficacy and adverse drug reactions. Busulfan has a narrow therapeutic range. High exposure is associated with systemic toxicity such as veno-occlusive disease and underexposure is associated with graft failure or relapse. In children, inter-patient pharmacokinetic variability was found to be high even after the use of intravenous (IV) busulfan. In this study, we identified biomarkers to predict busulfan pharmacokinetics using pharmacometabolomics. Therapeutic drug monitoring (TDM) was done for pediatric patients who underwent busulfan-based hematopoietic stem cell transplantation (HSCT). The 6-hours interval urine samples were collected before busulfan administration in 59 patients. The area under the curve (AUC) of busulfan was calculated and the subjects were classified into two groups based on AUC (Group 1; AUC <25,000 μg*h/L/day, n=50 and Group 2; AUC ≥25,000 μg*h/L/day, n=9). The urinary metabolite profiling was performed by ultra performance liquid chromatography time of flight mass spectrometry (UPLC-TOF-MS) in conjugation with multivariate statistical analysis. Principle components analysis (PCA) of urinary profiling was not separated between group1 and group 2 clearly, but group 2 tended to cluster together. The urinary metabolites associated with high level of busulfan AUC were identified to deferoxamine metabolites. Because deferoxamine was treated for patients with high ferritin level before HSCT, we analyzed the association between ferritin level and the 1st day AUC in 136 pediatric patients. The 1st day AUC/dose (mg/m2) was higher in patients whose ferritin level ≥ 1,000 ng/mL compared to that of the other patients (192.5 ± 56.5 μg*h/L vs 164.4 ± 40.0 μg*h/L, P=0.006). The optimal busulfan dose to meet the target AUC of 18,750 μg*h/L/day was 120.7 ± 28.1 mg/m2 in patients with ferritin < 1,000 mg/mL and 107.6 ± 28.0 mg/m2 in patients with ferritin ≥ 1,000 ng/mL (P=0.025). This study suggested the possibility of decreased busulfan metabolism in patients with iron overload or a possibility of drug interaction between busulfan and deferoxamine. Further study is needed to identify the exact mechanism of these results. The present study used pharmacometabolomics to identify biomarkers and metabolic mechanism implicated in prediction of busulfan pharmacokinetics.

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Assessment of Pharmacometabolomics of Drug-Induced Liver Injury after Administration of Amoxicillin/Clavulanic Acid in Human Subjects

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Drug-induced liver injury (DILI) is considered as one of the challenges when developing new drug and its application afterwards. Currently, alanine transaminase (ALT) and aspartate transaminase (AST) are widely used as biomarkers which represent the severity of liver injury. However, more sensitive and accurate biomarkers are still needed to predict the potential of DILI. Metabolites are “the end products of the cellular processes” and thus it can explain certain phenotypes, potential of DILI in this case, more precisely than other endogenous substances. In this study, we used pharmacometabolomics to evaluate potential biomarkers in the urine of healthy subjects after multiple administration of amoxicillin/clavulanic acid. An open-label, single-sequence study was conducted in 32 healthy male volunteers. Subjects received oral doses of amoxicillin 750 mg and clavulanic acid 375 mg twice daily for 14 days. The 12-hours interval urine samples were collected before and after drug administration on 0, 8, and 14th day. The urinary metabolite profiling was performed by ultra-performance liquid chromatography time of flight mass spectrometry (UPLC-TOF-MS) in conjugation with multivariate statistical analysis. Urine samples were diluted with distilled water and analyzed in both positive and negative mode of electrospray ionization. Using “MassLynx Mass Spectrometry Software”, principle components analysis (PCA), orthogonal partial least squares discriminant analysis (OPLS-DA), and s-plot were performed to select the candidates of biomarkers. A total of 31 subjects completed the clinical study and one subject dropped out due to headache and nausea. Subjects were classified into two groups based on fold change values of ALT on day 14(≥2.0-fold: Group 1; n=6, <1.5-fold: Group 2; n=16). According to multivariate statistical analysis, there were five urinary metabolites which concentrations were significantly changed after administration of drug on day 8. Those metabolites were identified to be 7-methylxanthine, 3-methylxanthine, acetylcarnitine, azelaic acid and 7-methyluric acid. Although the urinary metabolites level showed large inter-individual variation, 7-methylxanthine, 3-methylxantine and 7-methyluric acid were significantly up regulated while acetylcarnitine and azelaic acid were significantly down regulated. Among five identified metabolites, four were closely related to mitochondrial oxidative stress. Under the oxidative stress environment, expression of CES, azelaic acid producing enzyme, decreases by cell signaling which leads to reduction of azelaic acid. Xanthine oxidase is an enzyme which metabolizes methylxanthine to methyluric acid. This enzyme is known to produce reactive oxygen species (ROS), which leads to hepatocyte damage. In conclusion, four out of five metabolites identified in this study, Azelaic acid, 7-methylxanthine, 3-methylxanthine, 7-methyluric acid are known to be closely related to oxidative stress. These metabolites may be developed into early biomarker since urinary concentration of ALT changed on day 14 while urinary concentration of all five metabolites changed on day 8. Further confirmatory study must be performed including mechanism of these metabolites on drug induced liver injury. We evaluated and identified potential early DILI biomarkers based on currently known DILI mechanisms in this study.

poster 510

Specific biological response following USPIO administration

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Ultra-small superparamagnetic particles of iron oxides (USPIO) have been developed as organ/tissue-specific MRI contrast agents to improve the efficiency of magnetic resonance imaging in vivo, however, the overall biological effects and biochemical response induced by USPIO in the specific organ/tissue are still poorly understood, which should be closely related to their organ-specificity, dynamic process and metabolic fate in the body. Further, their specific biodistribution may make potential adverse effects. Such issues have attracted particular attention from the public and the scientific fields recently. In our study, 1H NMR spectroscopy integrated multivariate statistical analysis to investigate the biological responses of male BALB/C mice following dextran-coated USPIO intravenous administration. At the different time point post-dose, different biological compartments including plasma, urine, liver, kidney and spleen were obtained from control and USPIO-group mice. The metabolic variations were derived from the different biological compartments of the pair-wise mice groups. Hierarchical principal component analysis (H-PCA) trajectory demonstrated that the metabolic profiles of biofluids and tissues have obvious association with the biochemical process of USPIO in the body. At the 6 h post-dose, the metabolic information mainly involved in their absorption and transportation, and at 24 and 48 h post-dose, the metabolic activities

mainly involved in the biodistribution and secretion of USPIO. Furthermore, the detailed metabolic information of biochemical pathway and bio-related response were also derived from the OPLS-DA analysis of different biofluids and tissues metabolic matrices. Our results are helpful to understand the biochemical process of USPIO in the body and the subsequent potential adverse effects.

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Identifying individual differences of fluoxetine response in juvenile rhesus monkeys by metabolite profiling body fluids and skin fibroblasts

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Fluoxetine (Prozac®) is one of the first psychopharmacological agents approved by FDA for children and is commonly used therapeutically in a variety of developmental disorders. Therapeutic response shows high individual variability and severe side effects have been observed. In the current study we set out to identify biomarkers of response as well as biomarkers that correlate with impulsivity, a measure of reward delay behavior and potential side effect of the drug, in juvenile male rhesus monkeys treated daily with an oral therapeutic dose of fluoxetine or vehicle for one year. In addition to the commonly used peripheral body fluids, blood and cerebrospinal fluid, we have subjected fibroblast cultures from skin biopsies to metabolite profiling for affected pathway and biomarker identification. Juvenile male rhesus monkeys, genotyped for MAOA 5-HTTLPR polymorphisms, were treated daily with fluoxetine or vehicle and evaluated for behavioral functioning. Animals were sampled for blood, cerebrospinal fluid and skin biopsies for fibroblast culture. Metabolites were extracted from 50 ul plasma and cerebrospinal fluid and 107 fibroblasts with 80% methanol. Metabolite profiles were analyzed with a 5500 QTRAP LC-MS/MS system via selected reaction monitoring followed by Q3 peak area integration. A total of 302 endogenous water-soluble metabolites were detected. Metabolite/behavior associations were evaluated using multivariate analysis of metabolite intensities versus apical variables of each behavioral assay. We have interrogated three sample specimens. Though readily available, blood has the disadvantage of multiple sources of metabolites from different tissues and metabolic pathways. Although more suited as a source for brain studies, cerebrospinal fluid is obtained by spinal tap for collection, a rather invasive procedure not used in children for the evaluation of response to treatment on a routine basis. We therefore explored the use of an alternative specimen, skin fibroblasts, for assessing metabolite profiles. Skin fibroblasts can be retrieved by punch biopsy, which is less invasive and carries a much lower risk of infection compared to lumbar puncture. We hypothesize that brain metabolic pathways affected by fluoxetine treatment will also reveal themselves through metabolomics analyses in other cells of the body. Once fibroblast cells are established in tissue culture they have the added advantage of being removed from other environmental influences that are confounding metabolomics analyses in body fluids like blood and cerebrospinal fluid. Our analyses have identified metabolites that distinguish fluoxetine- from vehicle-treated animals and metabolites that correlate with impulsivity. The identified metabolite biomarkers belong to pathways that have important functions in central nervous system physiology. Affected pathway information will improve our understanding of adverse effects caused by fluoxetine in children. Biomarkers of response to fluoxetine in juvenile nonhuman primates as predictors of response to treatment in young psychiatric populations.

poster 512

Evaluation of CoA and Carnitine Metabolites in LIver and Serum from Mice Exposed to a Toxic Dose of Acetaminophen

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As a clinically important and therefore heavily studied toxicological model for investigating drug-induced liver injury, much insight has been gained into the mechanisms responsible for acetaminophen (APAP)-induced hepatotoxicity over the past several decades, yet many issues still remain unresolved. Advancements in analytical instrumentation together with improvements in computational methods have fueled the metabolomics field, and subsequently provided a powerful new platform by which APAPOSTER induced toxicologic events can be investigated. As a result, metabolomics has emerged as an invaluable technique for studying APAP toxicology. However, despite this rapid progress in analytical instrumentation and data analysis capabilities, the extraction and separation of metabolites, a critical step in any metabolomics study, has for the most part remained in a sub-optimized state. As the first and most critical of steps, it is of paramount importance that metabolite extraction and subsequent chromatographic separation be optimized to minimize any influence on data generation. To further investigate the effect of APAP toxicity on liver metabolism, comparative metabolomic analysis between control and APAPOSTER treated (400 mg/kg) C57BL/6J wild-type mice was performed using protocols optimized with regard to extraction efficiency and chromatographic resolution for acylcarnitines and coenzyme A thioesters, two classes of metabolites important for proper liver function and known to be impacted by APAP toxicity. A workflow for metabolite extraction (e.g., varying extraction solvent) and chromatographic separation (e.g., evaluating different column chemistries) optimization was developed and analyzed using a Waters UPLC coupled with a Xevo TQS triplequadrupole. For metabolite extraction, a variety of solvents were investigated including n-butanol, isopropanol (IPA), methanol, and mixtures of water, methanol, and chloroform. Further, the influence of pH was evaluated on the extraction efficiency. For acylcarnitines, acidified IPA (pH = 4) was found to be the most ideal extraction solvent for recovering acylcarnitines in liver and serum ranging from C0 to C18. Acyl CoAs were optimally extracted using a modified Bligh and Dyer extraction. Further, pH had a significant impact on overall extraction efficiency and therefore should be considered a critical component of any metabolite extraction optimization plan. Optimized chromatography was developed for both acylcarnitines and acyl CoAs and C18 BEH was found to provide baseline resolution. Interestingly, in terms of APAP toxicity, long chain acylcarntines (e.g., C14, C16, C18) and long chain acyl CoAs (C14, C16, C18) were significantly enriched in liver following a toxic dose of APAP thus supporting that 1) serum carnitine biomakers likely originate from the liver, and that 2) APAP significantly impacts mitochondrial fatty acid beta oxidation. Carnitines/CoAs are important metabolites for understanding APAP toxicity. Optimized tissue extraction and chromatographic separation is important for generating quality data.

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Metabolomics Evaluation of Preclinical Biomarkers of Hepatotoxicity: A Translational Update on Acetaminophen Toxicity Biomarkers

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Drug-induced liver injury (DILI) represents a major reason that drugs are recalled post market. Furthermore, it has been estimated that 10% of cases of acute liver failure are idiosyncratic in nature. Existing clinical markers of liver injury (alanine aminotransferase or ALT) are non-specific and relatively insensitive indicators of injury. New analytical platforms can be utilized to discover new omics-

based biomarkers that can be translated into a clinical setting. In order to identify biomarkers of hepatotoxicity, a systems biology approach was initiated to discover metabolomics biomarkers in blood from rats dosed with compounds associated with hepatotoxicity. Studies included examination of two overt hepatotoxicants, acetaminophen (APAP) and carbon tetrachloride (CCl4); two idiosyncratic hepatotoxins, felbatol (FEL) and dantrolene (DAN), and three drugs not typically associated with liver injury, meloxicam (MEX), metformin (MET) and penicillin (PEN). LCMS based open profiling metabolomics were applied to control, low dose and high dose samples from 7 studies in Sprague-Dawley rats. Focused metabolomics approaches and Biocrates kits were used in analysis of blood samples from clinical samples from APAP overdose and control patients. A PLS-DA classifier based on 6, 7 or 20 metabolites were 100% and 97.4% accurate at 6 h and 24 h for discriminating groups exposed to high doses of hepatotoxic compounds vs groups of rats exposed to control and high doses of the non-hepatotoxins (MET, PEN, MEL). This model was then used to predict liver toxicity of rats that were treated with idiosyncratic (DAN or FEL) hepatotoxicants at 6 and 24 h. One of the FEL rats was predicted to be toxic at 6 h while one of the DAN rats had a profile that was predicted to be toxic at 24 hr. The common metabolites observed in the DILI models at 6 h and 24 h were intermediates of fatty acid β-oxidation, bile acid and glycerophospholipid metabolism, which have been noted to be linked to the underlying mechanism of hepatotoxicity. Since idiosyncratic and non-hepatotoxicant drugs do not cause overt hepatotoxicity, doses were used to induce some adverse effect (e.g., a decrease in body weight) to provide a phenotypic anchor. Evaluation of some of the biomarkers observed in the nonclinical studies was evaluated in clinical samples from patients with APAP overdose. Elevation of several bile acid metabolites occurred in association with liver injury and elevations of APAP-protein adducts a marker of the oxidative metabolism of APAP. Importantly, several acyl carnitines were increased prior to the elevation of ALT (a translational standard test of liver injury) in rats and in some pediatric APAP overdose patients. Although the kinetic profile of specific biomarker and ALT elevation differed between rats and clinical samples, sequential relationships between these markers were similar. Acyl carnitines and bile acid metabolites are sensitive translational indicators of liver toxicity.

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Danazol is a substrate independent CYP2J2 inhibitor

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Cytochrome P450 2J2 is enzyme responsible for the metabolism of endogenous substrates including arachidonic acid as well as therapeutic drugs including albendazole, astemizole, ebastine, and terfenadine. To date, several CYP2J2-isoform selective inhibitors have been reported. However, the CYP2J2 inhibitory potential of these inhibitors has been evaluated for one or two CYP2J2 substrates. In this study, we evaluated the inhibitory potential of danazol, hydroxyebastine, telmisaratan, and terfenadone, representative CYP2J2 inhibitors, against CYP2J2-catalyzed albendazole hydroxylation, astemizole O-demethylation, ebastine hydroxylation, and terfenadine hydroxylation to find representative CYP2J2 inhibitor. The incubation mixtures containing cDNA-expressed CYP2J2, 0.1 mM phosphate buffer (pH 7.4), substrate (albendazole, astemizole, ebastine or terfenadine), and various concentrations of inhibitor (danazol, hydroxyebastine, telmisartan, or terfendone) were preincubated for 5 min at 37°C. Each substrate concentration was determined lower than reported Km value. The reactions were initiated by the addition of an NADPH-generating system and the reaction mixtures were incubated at 37°C in a thermo-shaker. After the 20 min incubation, the 50 μl of cold acetonitrile including the internal standard (IS; mebendazole) was added immediately to terminate the reactions. After centrifugation at 10,000 x g for 5 min, aliquots of the supernatant were analyzed by an LC-MS/MS. We evaluated the inhibitory potential of danazol, hydroxyebastine, telmisartan, and terfenadone which have CYP2J2 inhibitory potential for four representative CYP2J2 substrates, albendazole, astemizole, ebastine, and terfenadine using recombinant CYP2J2 enzyme. Of these four CYP2J2 inhibitors, danazol inhibited strongly CYP2J2-mediated astemizole O-demethylation, albendazole hydroxylation, ebastine hydroxylation, and terfenadine hydroxylation activities, with IC50 values of 0.07, 0.05, 0.34, and 0.18 μM in a substrate independent manner. Terfenadone showed strong inhibitory potential on the CYP2J2-mediated albendazole, astemizole and terfenadine metabolism, whereas it showed weak inhibitory potential on the

CYP2J2-catalyzed ebastine hydroxylase activity (IC50 = 6.04 μM). Telmisartan had no inhibitory effect on the CYP2J2-mediated ebastine and terfenadine hydroxylation (IC50 > 20 μM). The information from these data suggests that danazol may be used as a CYP2J2 index inhibitor in reaction phenotyping study.

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Development of screening method for five cytochrome P450 and four UGT enzyme activities using liquid chromatography-tandem mass spectrometry

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Cytochrome P450 (P450) and uridine 5′-diphosphoglucuronosyltransferase (UGT) are the representative phase I and II enzymes which play important role in the metabolism for most drugs, respectively. Several in vitro screening methods for the simultaneous evaluation of potential P450-mediated or UGT-mediated DDIs have been developed using cocktail incubation and tandem mass spectrometry. To date, however, the screening method for the simultaneous evaluation of P450 and UGT enzyme activities, which may accelerate evaluation procedure of DDI potential of new chemical entities (NCE) during drug development, has not developed. In this study, we report a new screening method that allows the simultaneous evaluation of the activities of nine human hepatic P450 and UGT enzymes (CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP3A, UGT1A1, UGT1A4, and UGT2B7). Human liver microsomal incubations contained either each substrate cocktail set (A set: phenacetin, diclofenac, S-mephenytoin, dextromethorphan, andmidazolam; B set: SN-38, trifluoperazine, mycophenolic acid, and naloxone) or an individual substrate. The cocktail doses and the concentration of each substrate were determined to minimize drug interactions among substrates. After termination of reaction, the supernatants of the individual reaction samples and pooled cocktail incubation samples (A set/B set, 1:1) were analyzed by LC-MS/MS. Known inhibitors of specific P450 and UGT enzymes (a-naphthoflavone, sulfaphenazole; S-benzylnirvanol; quinidine; ketoconazole; atazanavir; hecogenin; niflumic acid; and mefenamic acid) were incubated with each substrate cocktail set and with the individual substrates alone and the results were compared to validate developed screening method. We developed an LC-MS/MS method for the simultaneous determination of fiveP450 and four UGT enzyme activities. Nine substrates were divided into two cocktail sets for incubation and pooled for LC-MS/MS analysis in a single run. The inhibition potential of each P450 or UGT substrate was evaluated by comparing the reaction of each metabolite in the single substrate incubations, to the response of the same metabolite formed in incubations with the two-substrate cocktail set. The change in each P450 and UGT enzyme activity level was less than 15% in each cocktail set, compared with the individual incubation. The newly developed method as a screening tool for P450 and UGT inhibition was validated using known P450- and UGT-isoform selective inhibitors with their corresponding substrate. The inhibition curves show there was no substantial difference between the individual inhibitor profiles for the two different incubation methods (single vs. cocktail). The IC50 value of each cocktail set using this approach was comparable to those of the individual substrates and was in agreement with those previously reported. This confirms that the IC50 values of P450 and UGT inhibitors can be accurately determined using the cocktail assay instead of individual substrate incubations, which would save considerable time in the screening process for new chemical entities. This method offers a rapid and robust way to evaluate phase I and II enzyme inhibition profiles.

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Metabolic phenotyping of hepatic CYP3A-mediated drug interactions in both males and females

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Endogenous metabolic markers for CYP3A activity are useful in clinical applications of CYP3A substrate drugs to predict drug-drug interactions between in vivo CYP3A inhibitors and inducers. The aim of this study was to identify endogenous markers of hepatic CYP3A-mediated drug interactions in healthy subjects using a metabolomics approach. Endogenous metabolite profiles were determined using urine and plasma samples from 12 healthy female subjects, and a statistical model was generated to predict midazolam clearance (CL) using the endogenous markers associated with the inhibition and induction of CYP3A. The androstenedione and 11β-hydroxy-androsterone:androsterone ratios and 4-hydroxy-androstenedione:androstenedione ratios were selected as the final covariates for the model in females. The covariates that were selected for the equation differed between males and females, which suggests that the profiles of endogenous sex hormone metabolism differ between genders. We developed an integrated equation to predict midazolam CL that can be used in both genders.

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EVALUATION OF CYP2D6*10B ALLELE ACTIVITY THROUGH METABOLIC PROFILING USING LIQUID CHROMATOGRAPHY-MASS SPECTROMETRY.

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Metabolomics has recently gained popularity in fields related to the environment, plants, and clinical medicine. In clinical studies, metabolomics has been used to discover biomarkers and related metabolic pathways involved in human diseases, as well as to aid drug development, particularly for the prediction of responses and adverse effects. Cytochrome P450 (CYP450) is a heme-containing, major phase I enzyme involved in the metabolism of various drugs. CYP450 genetic polymorphisms are a major contributing factor to pharmacokinetic (PK) variation in drug responses. However, individual variation in the CYP2D6 genotype has not been well-defined using a pharmacometabolomic approach. In this study, individual variation in the CYP2D6*10B allele, a clinically important gene found in approximately 50% of Koreans, was evaluated by metabolic profiling. In total, 16 healthy Korean males were divided into CYP2D6*1/*1 and CYP2D6*10B/*10B using reverse transcription polymerase chain reaction, before being administered 100 mg metoprolol tartrate, a specific substrate of CYP2D6. Blood samples were collected for the measurement of blood concentrations of metoprolol and alpha-hydroxy metoprolol, and for the calculation of metabolic ratio (MR), using liquid chromatography-mass spectrometry (LC-MS). Metabolic profiling was conducted using LC-MS in urine samples collected 12 hours before and after drug administration. Multivariate analysis of the metabolic profile was performed using the SIMCA software package. The selected candidate metabolites were tentatively identified from the Internet databases METLIN, HMDB, and KEGG. The identification of metabolites was confirmed using authentic standard compounds. PK parameters, and the MR for CYP2D6*1/*1 and CYP2D6*10B/*10B, differed significantly among groups. The values for AUC0→∞ and Cmax of metoprolol, for the CYP2D6*1/*1 genotype, were significantly lower than those for the CYP2D6*10B/*10B genotype. MR was significantly higher for the CYP2D6*10B/*10B vs. CYP2D6*1/*1. Individual variation, with respect to MR, was similar in the CYP2D6*1/*1 (0.52 ± 0.17), but reduced in the CYP2D6*10B/*10B, genotype (4.57 ± 2.19). CYP2D6 enzyme activity inhibited the metabolism of metoprolol to alpha-hydroxy metoprolol in the CYP2D6*10B/*10B genotype. Multivariate analysis of urinary metabolic profiles was performed. In the principal component analysis score plot, outlier- and clustering-allowed visualization was not observed among groups (CYP2D6*1/*1, CYP2D6*10B/*10B, pre-dose, or post-dose), and quality control samples were tightly clustered with the other urine samples. Using partial least squares (PLS) modeling, 18 metabolites were identified and used to construct a metabolic network relative to tyrosine/phenylalanine,

tryptophan, purine, primary bile acid synthesis, and steroid hormone biosynthesis metabolism. To explore enzyme activity in CYP2D6*10B/*10B, which was characterized by marked individual variation, epinephrine, phenylalanine, and proline were selected by PLS modeling. For the selected metabolites, the evaluated MR for CYP2D6*10B/*10B correlated strongly with the actual MR. Using the correlation coefficients of the identified metabolites, an equation was developed for the CYP2D6 genotype, subsequently used to evaluate the individualized MR (MR = [3.35 - (0.55CON + 0.41EPI + 0.34PHE - 0.53PRO)] / 0.15). Therefore, our study demonstrates that a pharmacometabolomic approach can be used to evaluate CYP2D6 activity within the context of individualized, clinical drug therapies. The use of the metabolites allows us to predict the individualized PK before administering metoprolol and to monitor metabolic activities.

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Identification of affected molecular pathways in antidepressant responder/non-responder mouse model

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Drugs of the Selective Serotonin Reuptake Inhibitor (SSRI) type are commonly used antidepressants for the treatment of psychiatric diseases including Major Depression Disorder (MDD). However, a substantial number of patients do not show any improvement during or after antidepressant treatment. The high variability in response to SSRIs combined with a lack of clinically useful assessments that can reliably predict whether a patient will respond to a particular antidepressant compound prevent a strategic treatment and personalized medicine approach in psychiatry. Both, biomarkers predicting a priori whether an individual patient will respond to the treatment of choice as well as an early distinction of responders and non-responders during antidepressant therapy can have a significant impact to achieve this goal. Following one month of chronic Paroxetine treatment, DBA/2 mice were evaluated with the forced swim test (FST), an assay for depression-like behavior. Hierarchical cluster analysis of the FST data stratified drug responder and non-responder mice. Metabolites were extracted from the hippocampus, prefrontal cortex and plasma from each animal. Targeted metabolomics analysis was performed to identify metabolite profiles that distinguish Paroxetine-responder and non-responder mice and identify affected molecular pathways and biomarkers. Hierarchical clustering based on FST floating time identified two sub-groups, Paroxetine-treated short floating (PSF) and long floating (PLF) groups that served as proxies for drug responder and non-responder groups, respectively. Hippocampal metabolite profiles indicated a significant enrichment of pyrimidine and purine metabolism pathways. Pyrimidine and purine metabolite levels were significantly elevated in the PSF group compared to the PLF group and strongly correlated with treatment response/FST floating time. We also interrogated peripheral metabolome alterations by investigating plasma metabolites at baseline and four weeks after Paroxetine treatment from the same animal. Pyrimidine and purine metabolism pathways were again significantly enriched, this time in the plasma of Paroxetine responder mice. The expression of carbamoyl phosphate synthase 2 and hypoxanthine-guanine phosphoribosyltransferase 1, proteins involved in the pyrimidine and purine metabolism pathways, was different in the hippocampus and erythrocytes of the PSF and PLF mice upon chronic Paroxetine treatment, further supporting the involvement of the two pathways. Stratification of Paroxetine responder and non-responder mice by pyrimidine/purine metabolism pathway activity

poster 519

Metabolite profiling of plasma and leukocytes of chronic myeloid leukemia patients

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Chronic myeloid leukemia (CML) is a myeloproliferative disorder caused by constitutively active BCR-ABL tyrosine kinase. The objective of this work was to evaluate the influence of tyrosine kinase inhibitors (TKIs) approved in the CML treatment to the overall plasma and leukocyte metabolite profiles in the patients. Plasma and leukocyte samples were analysed by liquid chromatography (aqueous normal phase separation mode) coupled to tandem mass spectrometry (Qtrap 5500, AB Sciex, USA). The data acquisition was proceeded in both positive and negative multiple reaction monitoring mode and the metabolites were detected and quantified in MultiQuant 3.0 software. The statistical treatment of the data involved both unsupervised (principal component analysis) and supervised (discriminant function analysis, orthogonal partial least squares discriminant analysis) methods using R programme language with statistics packages. 152 metabolites in leukocytes and 124 metabolites in plasma were detected in 6 groups of samples: healthy controls, newly diagnosed patients, patients treated with Imatinib, Dasatinib, Nilotinib and Hydroxyurea. The unsupervised statistical models show overlap of hydroxyurea treated with newly diagnosed in both plasma and leukocytes samples suggesting similar metabolomic composition. In leukocyte samples TKI treated (except dasatinib) overlap controls whereas cluster far from newly diagnosed, which indicates metabolome regulation towards the normal profile. Although only four dasatinib treated patients leukocytes were processed, dasatinib treated partly overlap TKI treated, but seems to differ from controls. Also in plasma samples the TKI treated overlap controls, but the separation from newly diagnosed is not so clear as in leukocytes. The supervised statistical models show similar characteristics as the unsupervised ones with some trends more obvious. The changes in metabolite profiles of patients with good response compared to patients with poor response on treatment by imatinib were observed. Acknowledgements: CZ.1.05/2.1.00/01.0030, post doc grant CZ.1.07/2.3.00/30.0004, CZ.1.07/2.3.00/20.0170, Czech Science Foundation Grant I 1910-N26. First study dealing with investigation of plasma and leukocyte metabolome changes induced by CML itself and its treatment.

poster 520

A Biological Systems Analysis of the Arabidopsis thaliana Col-0 Wild Type Compared to Cold sensitive reil Mutants

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The Arabidopsis thaliana proteins REI1-LIKE (REIL1) and REIL2 are required for growth in the cold (10 oC), but not at optimal temperature (20 oC). A reil1.1 mutant revealed no phenotype differences when grown in cold, except for a slightly delayed germination. In contrast the paralogous allelic reil2 mutants, namely reil2.1 and reil2.2, developed small spoon-shaped leaves at 10 oC. The leaf shape reverted to normal after the appearance of an inflorescence in cold. The A. thaliana Col-0 wild type and the reil1.1, reil2.1, and reil 2.2 mutants were cultivated at 10oC in agar plates and transferred to soil after 4 weeks. Morphometric parameters of rosettes (number of leaves, leaf area, leaf diameter and surface coverage) were compared and the differences between rosettes before and after bolting at week 5 (w5) and week 7 (w7) after transfer to soil, respectively, were assessed. `Omics´-technologies were applied to compare the profiles of transcripts, lipids, primary, and secondary metabolites in vegetative and generative phases. Transcript analysis was performed using microarray technology. Metabolic profiling of primary metabolites was by gas chromatography-mass spectrometry (GC-EI-TOF-MS). Secondary metabolites and lipids were profiled by liquid chromatography-mass spectrometry (UPLC-FT-MS). The reil 1.1 mutants showed no significant differences of rosette diameter, number of leaves, and leaf area in both weeks when compared to wild type. In contrast, the reil 2.1 and reil2.2 mutants differed from wild type. The number of leaves, leaf area, and leaf diameter were smaller in both, w5 and w7. Surface coverage was higher in w5,

but similar to wild type in w7. Pearson's correlation matrices showed that reil1.1 and wild type transcripts have similar profiles. The two allelic reil2.1 and reil2.2 mutants were highly similar to each other but differed from both, wild type and reil1.1. These observations held true also for lipids, primary and secondary metabolites. The lipid profile, however, was the least changed among genotypes in w5. PCA analysis of the transcript and primary metabolite profiles as well as the lipid and secondary metabolite profiles indicated that the reil2.1 and reil2.2 mutants in w7 are more similar to wild type in w5 rather than to wild type in w7. The results suggest that reil2 mutants are growth-arrested compared to WT. Primary metabolite analyses indicated that reil2 mutants accumulate sugar and organic acids before bolting (w5) but not after (w7) when the inflorescence grows. Amino acids were less abundant in w5 than in w7 compared to WT. Transcript analysis suggests an increased protein metabolism after bolting, since categories of genes related to amino acid synthesis and protein degradation were preferentially up-regulated in w7. Future functional assessment of the differential transcripts and metabolites will give a hint of the nature of the delayed development caused by the absence of a functional REIL2 gene and will link the mutant phenotypes to the poorly investigated vegetative to reproductive transition in the cold. Metabolic and Transcript profiles indicate that reil2 mutants may be physiologically younger than WT before bolting at 10 oC.

poster 521

Obtaining Wide Metabolome Coverage through Multi-platform Profiling of a Single and Minute Plant Sample

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It is currently well established that obtaining wide coverage of the metabolome in any biological sample requires the use of a multi-platform approach enabling the detection of metabolites possessing diverse polarities. The use of several profiling methods for analyzing different replicates of the same samples typically decreases the reproducibility of the data obtained. This is even a greater issue when analyzing samples for which only a limited and minute amount of material could be collected. To allow multi-platform profiling of single samples having tiny amount we have developed a strategy combining UPLC-QTOF-MS, UPLC-QqQ-MS and GC-TOF-MS instruments.This approach enabled us to detect primary (polar) and secondary (semi-polar) metabolites as well as phytohormones and lipids belonging to diverse classes from a single tube preparation. In the presentation, I will demonstrate the use of this strategy to perform extensive profiling of tomato extracts, including the analysis of the microscopic, approximately 100 micrometer in length meristematic zone, prior and after the transition to flowering. Forty to 80 dissected meristems were sufficient for profiling each sample that contained not more than 1mg fresh tissue. Major differences were detected between the vegetative and floral meristematic tissue, providing the first insight to the unique metabolic features of this vital plant tissue. Mass spectrometry imaging could also be of great value in metabolic analysis of samples containing low total mass. Consequently, I will also present recent experiments employing MALDI-FTICR-MS to map the spatial distribution of metabolites in apical plant meristems. This study represents a wide metabolome converging through multi-platform profiling of a single and minute plant sample.

poster 522

Unrevealing the unique metabolic features of meristematic cells at the vegetative and floral stages through high-resolution mass spectrometry-based lipidomics

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The plant meristem contains undifferentiated cells (meristematic cells) that generate new organs as the plant develops and matures. Our lab is interested in studying the unique metabolic features of this tiny

zone (approx. 100 micrometer in length) which is likely very active metabolically and requires constant protection from biotic and abiotic stresses. Hence, we have dissected apical vegetative meristem tissue (forming leaves and stems) from young tomato plants and those at later stage in which meristems have already transitioned to flowering and generated floral organs. The minute amount of meristem tissue per sample (<1 mg) was subsequently used for lipidomics assays using high-resolution UPLC-q-TOF-MS. Lipids were extracted by a mixture of MTBE-methanol and the organic phase was concentrated and analyzed in the MSE mode comprising low and high energy scan functions to acquire accurate mass data for intact precursor and product ions, respectively. Overall, more than 275 individual lipid molecular species were detected in tomato meristems, including galactolipids, sphingolipids, phospholycerols and glycerolipids. Principal component analysis suggested that the vegetative and floral meristems lipid profiles were clearly different. Compared with meristems in the vegetative stage, PCs, lyso-PCs, lyso-PEs, TAGs, acylated steryl glucosides, and long-chain (34 or more carbon atoms) PE, MGDG, DGDGs and DAGs were significantly increased in the floral stage. On the other hand, some short-chain (32 or 34 carbon atoms) PEs, MGDGs, DGDGs and DAGs were significantly decreased in floral stage. Interestingly, DAGs produced in the vegetative meristems were mostly saturated as compared to the floral ones producing predominantly unsaturated. This study represents the first attempt to dissect the unique metabolic features of important zone for plant growth and maturation.

poster 523

Dry and Determined: Constraints On The Anhydrobiotic Metabolome in Ten Desiccation Tolerant Plants

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Desiccation tolerant (resurrection) plants can survive the loss of over 95% of cellular water from vegetative tissues, and regain full metabolic function on rehydration. This trait, although rare among higher plants, is observed among clades as diverse as mosses, ferns and fern allies, and several angiosperm families, both mono- and dicotyledonous. In all of these cases, the dry state is associated with the abundant accumulation of various small molecular species within the cell, and depletion of others, with the effect of stabilizing macromolecules and cellular structures in the absence of water. This study aims to generalize observations about desiccation-related metabolome changes and work toward a comprehensive model of chemical constraints associated with retention of viability in the anhydrobiotic state. Ten species of resurrection plants, ranging from fern allies to more recently evolved species,were surveyed for changes to the primary leaf metabolome on drying. Leaf samples were excised from three individuals of each species, both while fully hydrated and after desiccation to the air dry state. Tissue samples were ground under liquid nitrogen and lyophilized before extraction of polar metabolites with 50% methanol. Vacuum-dried metabolite extracts, as well as standards of various sugars, sugar alcohols, amino acids and other organic acids, were derivatized with methoxyamine hydrochloride and MSTFA, and analyzed by GC-MS. Peak areas were normalized to a ribitol internal standard, and quantified against standard calibration curves. Quantitative data were analyzed by PCA and PLS-DA to identify compounds of interest. PCA visualization of the data set revealed that the metabolome present in anhydrobiotic states were less variable than that associated with hydrated, metabolically active states. In general, comparable changes in metabolite abundance were viewed across species, including the depletion of reducing monosaccharides, the accumulation of sucrose and, in several cases, dramatic changes in the abundance of small organic acids such as malic and citric acids. Despite differences among species in the specific changes observed, the patterns observed showed significant commonalities. It is suggested that the relative abundance of specific compounds, or classes of compounds, in the dry state must be maintained in tightly defined ranges in order to achieve successful stabilization of membranes, macromolecules and cytoskeletal elements during anhydrobiosis. A survey of primary metabolomes in a broad range of resurrection plants, with insight into chemical constraints associated with anhydrobiosis.

poster 524

Metabolite profiling of a variety of tea leaves by GC/MS

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In recent years, an variety of teas such as persimmon (Diospyros kaki Thum b.) tea, lotus (Nelumbo nucifera) tea, guava (Psidium guajava) tea, mulberry (Morus alba L.) tea, mint (Mentha arvensis var. piperascens) tea, bamboo (Phyllostachys nigra) tea was developed in addition to traditional green tea (Camellia sinensis L.) and became to be popular among people. The aim of the current study was to investigate the pattern and difference of metabolites of those various tea leaves through targeted profiling of 312 metabolites by GC/MS and SIMCA-P. Dried tea leaves were cut and homogenized using a mortar & pestle with liquid nitrogen. 1.5ml of 80% Methanol (4°C) and 60ul of Ribitol (0.2mg/ml) as Internal standard were added to 100mg of ground tea before extraction (15°C, 10min) and centrifugation (4°C, 13,000rpm, 10min). Chlorofilter® (50mg) was added to 1 ml of supernatants, voltexed (10s), centrifuged (4°C, 13,000rpm, 10min) and supernatant was evaporated using Speed-Vac®. The residue was derivatized with methoxyamine hydrochloride (40ul, 20mg/ml in pyridine) and with MSTFA (1% TMCS, 70ul). Aliquot (1 ul) was analyzed by GC/MS (Shimadzu 2010). The 312 components of targeted analysis consist of various ketones, alcohols, aldehydes, amides, amines, amino acids, amino sugars, antibiotics, monosaccharides, disaccharides, fatty acids esters, fatty acids, fatty alcohols, lactones, inorganic acids, nucleobases, nucleosides, nucleotides, organic acids, phosphates, purines, steroids, sugar acids, sugar alcohols, ureas and etc. Some sugars such as sorbose, fructose, glucose, galactose, tagatose, allose, psicose, trehalose, sucrose were observed to be relatively high in persimmon, lotus, guava, and mint teas. Also Some organic acids including threonic acid, malic acid, citric acid, isocitric acid and succinic acid were high in persimmon, guava, mulberry and bamboo teas. Caffeine was detected only in green tea while inositol was absent in bamboo. Meanwhile, amino acids such as valine and phenylalanine were present in lotus, mulberry and mint tea more than in bamboo, green and persimmon teas. Those 7 different tea leaves were discriminated successfully on PCA according to their metabolites patterns. In order to remove chlorophylls in tea extracts, a solid sorbent Chlorofilter® was used instead of conventional chloroform.

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Sightsee the nutrition needs of Citrus Huanglongbing pathogen, Candidatus Liberibacter asiaticus using comparative metabolomics of Citrus phloem sap

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Candidatus Liberibacter asiaticus (CLas) causes citrus greening disease or Huanglongbing (HLB). The phloem-restricted bacterium, CLas, is vectored by the Asian citrus psyllid (ACP),Diaphorina citri, a phloem saPoster feeding insect. We studied the composition of the citrus phloem in an effort to understand the cultural requirements of this fastidious bacterium. Plant phloem sap is an aqueous solution of mostly sugars, as well as other organic and inorganic compounds. Phloem sap samples were collected from HLB-tolerant citrus varieties such as Citrus latipes, Poncirus trifoliate, lemon and limes, and HLB-susceptible cultivars such as Citrus sinensis ‘Valencia’ grapefruit and Citrus macrophylla. Samples were derivatized using a two-step trimethylsilylation (TMS) for GC-MS, which detects organic compounds, especially sugars. Using this method, we identified and quantitated from citrus phloem sap, 60-90 compounds that included amino acids and amines, organic acids and carbohydrates We also used methyl chloroformate derivatization (MCF), which is very sensitive and specific for amino acids and organic acids which allowed us to detect and precisely quantify amino acids. Although the overall composition of the

phloem sap was similar between susceptible and tolerant citrus varieties, the metabolite profile was unique for each variety, with some trends that correlated with tolerance to HLB. Glucose concentration was higher in the phloem sap than sucrose in 'Valencia' and Poncirus trifoliata, but sucrose was higher than glucose in susceptible variety 'Valencia' which also contained raffinose in significant amounts. C. latipes, had the most L-proline and the highest concentration of amino acids. Carbohydrates were highest in two grapefruit varieties, lemon and lime. Organic acids were lowest in 'Valencia' and P. triofoliata and highest in Citrus macrophylla. We found that tolerant varieties contained elevated amino acids levels, especially phenylalanine and tryptophan and tyrosine, in response to biostress. Sugar alcohols known to support growth of Gram-negative bacteria were found in abundance in citrus phloem sap, which suggested that they might be required for CLas proliferation The comparison of phloem saps revealed differences potentially could be important in understanding the nutritional requirements for CLas bacterium

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Systematic and Biological Driven Annotation of Metabolites in the Model Legume Medicago truncatula using UHPLC-QToFMS-SPE and NMR

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Large-scale profiling of plant metabolites helps to advance our fundamental understanding of plant biochemistry and yields discovery of novel metabolites and gene functions. However, the depth-of-coverage of identified plant metabolites is estimated to be between 10% and 20%. We have addressed this limitation by developing a UHPLC-QToFMS-SPE-NMR platform which facilitates and accelerates the systematic identification of the metabolites in the leading model legume, Medicago truncatula. This platform has been used to confirm the chemical structure of over thirty metabolites in Medicago truncatula, some of which are reported here for the first time. As a result, the biological context of our metabolomic experiments are increasing, thus providing even greater opportunities and understanding of this model legume. Systematic and biologically driven metabolite identifications were pursued using an UHPLC-ESI-QToFMS-SPE-NMR platform. For this study, aerial and root tissues of Medicago truncatula were extracted and analyzed. An efficient, automated UHPLC-ESI-QToF-SPE method was developed for the isolation and concentration of targeted compounds, thereby, enabling higher-throughput structural identification by NMR analyses. Accurate mass measurements, isotopic ratios, elemental formulas, and MS/MS spectra from UHPLC-ESI-QToF-MS are being used along with in silico software (PlantMAT) to provide putative identifications of metabolites. Targeted UHPLC chromatographic peaks were isolated, trapped and concentrated using automated SPE. Aided with the putative identifications provided by PlantMAT, full compound structural confirmation of the isolated compounds was done by 1D and 2D NMR analysis. Although routine LC/MS analysis of aerial/root tissue from Medicago truncatula can be accomplished using 10 mg of dried tissue, a mass of 100-1000 mg was needed to provide sufficient isolated/purified compound (>1µg) for NMR analysis. For automated SPE, it was determined that Waters Oasis HLB (hydrophilic/lipophilic balance) cartridges (1 mm x 10 mm) yielded the best recoveries. The HLB cartridges were eluted with 250 µL of methanol-d3. The entire eluent from the SPE cartridge was collected in autosampler vials and the solvent evaporated to a volume of 30 µL before transfer to 1.7 mm o.d. NMR tubes. The average solid phase extraction recovery of this method was determined to be 93% for nine flavonoids and saponins. The work-flow for compound identification in plant tissue extracts consists first of dereplication of those chromatographic peaks that can be identified from comparison with mass spectra already present in our MS and MS/MS libraries of authentic, putative, and NMR confirmed compounds. Then, unidentified chromatographic peaks were targeted depending on their biological significance. As many as twenty-five replicate injections of the tissue extract were concentrated on individual cartridges for each targeted peak. A UHPLC mass chromatogram of an extract of aerial Medicago truncatula tissue will be provided with peaks annotated with the NMR confirmed structures including over thirty polyphenolic glycosides, saponins, phospholipids, and fatty acids. UHPLC-ESI-TQoFMS-SPE-NMR methods provide full structural confirmation and increased depth-of-coverage of metabolites in aerial/root extracts of Medicago truncatula.

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Across-Instrument (UHPLC-QToF-MS) Evaluation of Mass Spectral Libraries for Identification and Dereplication of Plant Metabolites in Aerial/Root Extracts of Medicago truncatula

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The annotation or identification of metabolites is critical to understanding the biological context of metabolomic data. An initial step in annotation involves dereplication, or the identification of those metabolites in a sample that have already been identified. In LC/MS this is done by comparing the mass spectra of chromatographic peaks in the sample with the mass spectra of known compounds in mass spectral libraries. We have constructed mass spectral libraries of authentic standards, and NMR confirmed and putatively identified compounds found in the aerial/root extracts of Medicago truncatula (MT). To evaluate the across-instrument utility of these libraries for compound identification, we compared the search results from two different UHPLC/QToF/MS instruments which analyzed an identical extract of aerial/root MT tissues. MS and MS/MS libraries were constructed from the UHPLC/QToF analyses of 222 authentic compounds, and 61 NMR confirmed and 40 putatively identified compounds from aerial/root extracts of Medicago truncatula. A second UHPLC/QToF system having an identical QToF MS (Impact HD, Bruker Daltonics) but a different LC was used to analyze the same extract. A Waters Acquity BEH C-18 UHPLC column (150 mm x 2.1 mm x 1.7 µm) and the same LC conditions were used in both systems. The retention times in the MS libraries were adjusted for systematic differences between the two UHPLC/QToF systems by applying retention time corrections found from the analysis of a mixture of reference compounds. Library search results for the two LC/MS systems were compared. LC/MS chromatograms of an extract of aerial/root tissue of Medicago truncatula analyzed on two different UHPLC/QToF systems will be provided and will show a retention time offset of about one minute. This offset is probably due to a difference in the dwell volumes (~0.58 mL) between the two liquid chromatographs. In order to include retention time as a parameter in the library search routine, a retention time alignment in the MS libraries was done by applying a correction determined by analyzing the same calibration mixture on both systems. This mixture contained twenty flavonoids, soyasaponins, and unsaturated carboxylic acids which eluted throughout the LC gradient. A scatter plot of the retention times of these compounds between the two systems will show excellent linearity and a nearly perfect slope of 0.996. This is an indication that the gradient fluctuations are essentially the same in the two systems. The offset from zero in the plot, 1.03 min, is an indication of the dwell time difference. The retention times in MS libraries used for searching compounds in the second UHPLC/QToF system were adjusted for this difference. Injections of duplicate aerial/root MT extracts were analyzed on both UHPLC/QToF systems and the library search results were compiled. Tables will be provided that show how well the results obtained in the second system compared to the first system where the RT and MS spectra were originally obtained. Mass spectral libraries were used on different instruments to successfully annotate plant metabolites found in aerial/root extracts of M. truncatula.

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Discovering the metabolic changes of legumes exposed to combined biotic and abiotic stress over time.

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The yield of legume crops is severely undermined by both drought and fungal infection. A current EU FP7-project (Abstress) is using state-of-the-art plant breeding tools alongside the study of gene and metabolite expression data in legume species to study their resistance mechanisms to combined stresses. Metabolomics contributes to the understanding of the expression of crucial hub genes

associated with combined resistant traits, significantly reducing the time taken to develop new crop varieties able to withstand the challenges associated with climate change. Metabolite inheritance patterns can be used to determine the success of improving resistance to stress throughout the breeding cycle. Implementation of measurement techniques relating to metabolites can directly and indirectly measure phenotypic traits such as disease and drought resistance. To identify metabolite patterns associated with combined stresses metabolite data has been acquired using LC-HRMS in both roots and leaves for the model legume Medicago truncatula. Plants were grown in controlled greenhouse conditions and sampled each day over 15 days for 4 test groups: a)drought stressed, b)Fusarium infected, c)both drought and Fusarium infected and d)no stress applied. Each sampling day had 4 replicates per group. Metabolite data was normalised using QC responses after analysis by ProgenesisQI for alignment, peak picking and basic statistical assessment. Further statistical analysis included clustering (k-means) and Pearson correlation to identify metabolite patterns of interest across the time course study. Once tentatively discovered, compound identification and confirmation were undertaken using LC-HRMS/MSn. The data acquired in this study offers a good example of unintended batch bias over a 7 day LC-HRMS analytical time frame that can be accurately corrected using simple in-house normalisation procedures. The importance of a robust QC set for these procedures will be discussed. Furthermore, correlation and clustering methods have been evaluated to identify changing metabolite patterns over a time course study. In both roots and leaves a number of biologically associated metabolites have been identified as changing over time (i.e. compared to control plants) in each of the single stress groups. These include compounds already discussed in the literature regarding drought stress, such as proline, ABA, glutathione and organic osmolytes. More interestingly, using the cluster and Pearson correlation analysis, a number of metabolites have been identified with unique expression patterns over time in the dual stress plants compared to the other single stressors or control plants. These compounds include flavonoids and metabolites associates with lipid metabolism. Some of these are still only tentatively identified. Results from corresponding transcriptomic analysis will help to confirm tentative biological conclusions. Metabolite changes induced by combined biotic and abiotic stress in maturing legume plants. Methods to interpret time-course metabolomics experiments.

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Stable isotope assisted plant metabolomics reveals novel insight into the interaction of Fusarium graminearum and wheat

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The mycotoxin deoxynivalenol (DON) is produced by the fungus Fusarium graminearum and acts as a virulence factor of the devastating Fusarium head blight disease on wheat. Affected host plants can partly escape this eukaryotic protein translation inhibitor by conjugating and sequestering it to the vacuole or cell wall polymers. Despite its essential role for host plant colonization, little is known about the effects of DON on host plant metabolism and defense related processes. Here we use U-13C DON, U-13C phenylalanine and U-13C labeled wheat together with our LC-HRMS workflows for the untargeted profiling of i) toxin metabolism, ii) the effect of DON on phenylalanine derived metabolites in cell culture and iii) the global metabolic response of wheat upon DON treatment. Biological experiments involved treatments of flowering wheat ears with mixtures of native and U-13C DON, native DON plus U-13C phenylalanine or native DON in case of 12C and U-13C labeled wheat (enrichment >98%, in-house production in 13CO2 labeling chamber). After harvesting, quenching and sample preparation, measurements involved mixtures of labeled and native samples. C18 reversed phase HPLC was coupled to an LTQ Orbitrap XL in full scan mode. All raw data were evaluated with MetExtract II. This novel in-house developed software supports isotope assisted metabolomics and has been used successfully for

the untargeted screening of all tracer (13C DON or 13C phenylalanine) derived metabolites or alternatively the global metabolic composition of wheat ears after treatment with DON. In treated wheat ears, DON detoxification occurred via two major metabolism routes, glucosylation to DON-3-glucoside, DON-di-hexoside, 15-acetyl-DON-3-glucoside, DON-malonylglucoside and glutathione conjugation to DON-S-glutathione, "DON-2H"-S-glutathione, DON-S-cysteinyl-glycine and DON-S-cysteine. Based on our powerful isotope assisted approach, a total of nine different DON biotransformation products were found, eight of which were found here for the first time. Complementary to this, co-incubation of wheat cell suspension cultures with U-13C phenylalanine and native DON resulted in the detection of as many as 139 phenylalanine derived metabolites. The tracer origin of each of these metabolites was successfully verified via its corresponding unique labeling specific isotope patterns. Interestingly, the benefit of fast polarity switching was evident, with 32 and 58 of these metabolites having exclusively been detected in the positive and negative modes, respectively. Moreover, supported by the knowledge of the number of tracer derived carbon atoms, ca. 50 metabolites were successfully annotated as defense related phenylpropanoids, coumarins, phenylpropanoid amides, flavonoids and phenolic acids. Statistical evaluation showed that treatment with DON increased or decreased the abundances of many of the detected metabolites significantly. Finally, global metabolite profiling of mixtures of native and 13C labeled DON treated wheat ears revealed more than 1000 metabolic features corresponding to ca. 500-600 true wheat metabolites. Different metabolites were observed between control and DON treated plants underlining the extra benefit of equally treated native and labeled plants for the discovery of pathogen induced metabolites. Interestingly, the metabolic route, velocity and extent of DON-induced metabolism differed significantly among the tested wheat lines. Under the tested conditions, DON was clearly demonstrated to be a potent elicitor of many defense related metabolic processes in wheat. 13C labeling and isotope assisted fully automated LC-HRMS data processing enables significantly improved metabolomics of plants and their (a)biotic interactions.

poster 530

Metabolic profiling of flaxseed (Linum usitatissimum) depending on climatic conditions.

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Compounds study of flaxseed (Linum usitatissimum) arouses a growing interest in the last decade. Indeed, flaxseed contains large metabolites amountsthat can be valued in different industrial sectors. Lignans, for example, have an interest in the pharmaceutical industry for their anti-oxidant and anti-cancerous properties. The mucilage contained at the external layer of the seed coat has viscosity and hydrophilic properties that can be exploited in cosmetics processes. Thus, it is necessary to get informations on the metabolites content in mature flaxseed and possible variations depending on the culture conditions during seed development. The present study concerns the composition of different varieties of oilseed flax grown in different regions in France between 2011 and 2014. 1H-NMR was used to achieve a non-targeted metabolic profiling. Lignans as secoisolariciresinol diglucoside (SDG), caffeic acid glucoside (CaAG), Poster coumaric acid glucoside (CouAG) and ferulic acid glucoside (FeAG) were specifically analyzed by reversed-phase high pressure liquid chromatography (RPOSTER HPLC-UV). The monosaccharide composition analysis of mucilages obtained by soaking seeds was performed using high-performance anion exchange chromatography coupled with pulsed amperometric detection (HPAEC-PAD). Multivariate analysis were performed taking into account climate conditions such as precipitations, sun, growing degree days and soil parameters. Results show that soil and climate conditions seem to play a role on the flaxseed content of certain metabolites such as polyphenols or cyanogenic glycosides. The yield of mucilage and its sugar composition also seem affected by the climatic conditions. Thus, some metabolic pathways appear to be modulated and influenced by climatic conditions. Metabolomic is an efficient tool to analyse the impact of climate changes on the mature seed compounds.

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The emerging barley seed metabolome studied by mutant analysis: Effects of development stage, genotype and growth temperature

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The immensely complex plant metabolome is dynamically emerging through the developmental stages during epigenesis of the seed. Metabolome of growing plants and plant food products are influenced by environmental and genetic factors. Recent progresses in metabolomics allow quantification of effects of such factors on plant food products and may reveal new insight into plant-genotype-environment interactions through dynamic changes of metabolites. This study demonstrates a gene specific metabolomic analysis of barley endosperm seed model including two mutant genotypes, low-starch-high-β-glucan (lys5.f), and high lysine (lys3.a) and their isogenic mother line (Bomi) grown under different temperatures. The three barley genotypes were grown at 15 and 25 °C in a climate chamber under controlled conditions and seeds were analysed by GC-MS metabolomic profiling approach at eight developmental stages during the grain filling period. Raw GC-MS data obtained for all barley samples were processed by an advanced multi-way modeling technique, PARAllel FACtor Analysis 2 (PARAFAC2), and 247 peaks were deconvoluted and quantified. Of 96 peaks were identified based on EI-MS and RI library search and included phenolic acids, aldehydes, esters, organic acids, alcohols and fatty acids. Obtained 'full-design' metabolomics data facilitated the use of study design during unsupervised and supervised multivariate data analysis to investigate development of barley seed metabolome and factors influencing it. PARAFAC2 allowed high-throughput quantitative and qualitative analysis of complex raw GC-MS data without prior data treatment and resulted in a complete metabolite table. Application of ANOVA-Simultaneous Component Analysis (ASCA) revealed the main effects of study design factors, development stage, barley genotype and growth temperature, and their interactions on seed metabolome. We found three dominating metabolomic patterns during the seed development, common throughout the all genotypes. In addition, some organic acids exhibited genotype specific dynamics and increased in one genotype and decreased or remained status quo in the two other genotypes. This was in agreement with only observed two-factor significant interaction effect, between genotypes and development stages. The study further revealed the presence of signature metabolites for the investigated three barley genotypes. Global correlation tables between the metabolites at different developmental stages revealed a significant deregulation of the metabolome for the two mutants compared to Bomi and a significant deregulations due to high temperature that were more pronounced in the mutants. Genotype specific metabolite correlation tables also suggested an effect of growth temperature and depicted temperature dependant changes in correlations among metabolites. This effect further proved by ASCA showing a significant temperature effect. However, metabolites highly influenced by the growth temperature were not genotype dependant, but common for all thee barley lines. The use of study design and advanced chemometrics to investigate gene modification, growth temperature and development stage on barley metabolome.

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Insights Into the Duckweed Metabolome; a Fast Growing Free Floating Aquatic Monocot

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The aquatic plant Lemna, commonly known as duckweed, is the smallest, fastest growing and morphologically simplest of flowering plants. It is a monocot, closely related to crop plants such as Musa musa (banana) and Zea mays (maize). Presently, Lemna has a myriad of applications for e.g., waste water treatment, biofuel production, and even as a food source for animals and humans in the Far East.

Recently the genome of one member of the Lemnaceae family, Spirodela polyrhiza was fully sequenced. Due to its unique life cycle and together with available multi-omics datasets, as the comprehensive metabolic-map presented here, Lemna is an ideal model system for the study of monocot metabolic networks, including genome-wide metabolic model reconstruction and 13C flux analysis. Lemna metabolic profiling was performed using high resolution QTOF mass spectrometry (QTOF-MS) coupled with Ultra high performance liquid chromatography (UPLC), gas chromatography coupled with mass spectrometry (GC-MS) and HPLC with PDA/Fluorescence detection. Dependent on the chemical nature of the analytes various extraction methods, e.g. Bligh & Dyer, acidic methanol and others were optimized for Lemna samples in order to gain maximum coverage of the Lemanceae metabolome. 13C-Flux modeling was performed using 13CFlux2 followed by matching to results from feeding experiments. Data analysis, i.e. comparison between different species and relative peak quantification or quantification of isotopomers was done with Xcalibur, Masslynx and XCMS for R software and in-house tools for statistical analysis. In order to study the Lemnaceae metabolome, particularly secondary metabolism, extensive metabolic profiling was performed with four members of the duckweed family, namely Lemna gibba, Spirodela polyrhiza, Landoltia punctata and Wolffia globosa. Using GC- and LC- MS and UV/Fluorescence detectors a myriad of different substances could be identified from various compound classes, e.g. 291 lipids, over 50 flavonoids, 40 primary metabolites and 25 terpenoids. Besides putative identification using high accurate mass and MS/MS fragmentation patterns we performed targeted analysis in which metabolites were identified by comparison to authentic standards and to an in-house generated LC/MS reference library. Searching against the in-house LC-MS library resulted in unambiguous identification of 31 secondary metabolites from 8 different natural product classes. This metabolome dataset represents the first comprehensive study on Lemna's metabolism and can be used to identify potential “high-value” specialized metabolites as well as provide the base for the identification and characterization secondary metabolite pathways in Lemna. Complementary studies performed at present include genome-scale metabolic model reconstruction and the examination of dynamics of Lemna metabolism using 13C Flux analysis. Together these studies will serve as a basis for conducting predictive metabolic engineering to increase nutritional quality and/or yield of potential biofuel compounds as it was recently shown for the accumulation of multiple antioxidant caffeic acid derivatives in genome duplicated strains of Landoltia punctata. -First comprehensive study of Lemna metabolome -31 unambiguously identified secondary metabolites -Study of metabolic flux in a “whole plant” system

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Unravelling insect detoxification mechanisms of plant secondary metabolites– Identification of metabolite profiles in M. sexta

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Exploring the diversity of plant secondary metabolites is challenging and requires efficient methods to gain sufficient structural insight to quickly identify known compounds and enable identification of unknowns. De novo structure elucidation remains a major bottleneck, and improvements in the systematic analysis of MS fragmentation patterns of metabolite classes with complex decorations are needed to facilitate high-throughput compound annotation. 17-hydroxygeranyllinallool diterpene glycosides (HGL-DTGs) are abundant defensive secondary metabolites whose malonylation and glycosylation decoration steps are regulated by the jasmonic acid pathway in the model plant Nicotiana attenuata [1]. Here, we compare frass-specific HGL-DTG profiles of larvae of the lepidopteran herbivore Manduca sexta fed with N. attenuata plant material deficient in several biosynthetic steps of HGL-DTGs to unravel their defensive function. Leaf tissue from stable genetically silenced lines of N. attenuata impaired in geranylgeranyl diphosphate synthase (Naggpps), glucosyltransferase 1 (Nagt1), 1/2 (Nagt1/2) and rhamnosyltransferase 1 (Nart1) and frass of 2nd instar M. sexta larvae were extracted with 80% methanol. Chromatographic separation of extracts was carried out using a Dionex UltiMate 3000 Rapid Separation LC System combined with an Acclaim RSLC 120 C18 2.2µ 120 Å (2.1 × 150 mm) column. MS analysis was performed using a high-resolution QTOF (micrOTOF-Q IIBruker Daltonics) MS

detection system in electrospray positive ion mode. Deconvolution of In-Source CID (IS-CID) clusters was performed using the Dissect algorithm and molecular formulae were determined by SmartFormula 3D. GL-DTGs are diverse plant secondary metabolites with various sugar and malonyl moieties. Containing multiple labile glycosidic bonds, HGL-DTGs exhibit extensive in-source fragmentation (IS-CID), which provide valuable information for structural elucidation. Using a deconvolution algorithm, we reconstructed IS-CID clusters and identified precursor ions. Those ions were selected to acquire MS/MS spectra and create an MS/MS database that formed the basis to generate a set of rules for a fast dereplication of this compound class. This method allowed a rapid classification of different HGL-DTG`s in leaf material as well as frass. We detected diverse effects while analyzing the metabolite profiles in genetically silenced lines of N. attenuata impaired in the biosynthetic steps of HGL-DTGs. We found reduced levels of HGL-DTG concentration in Naggpps and Nagt1/2. We detected a much simpler metabolite profile in Nart1, due to the complete loss of rhamnose in the metabolites. In Nagt1 we discovered an enrichment of intermediate biosynthetic products and high levels of the aglycone 17-hydroxygeranyllinalool. Additionally, we performed feeding assays and found that larvae of M. sexta gained up to ten times more weight in Naggpps than in WT. Naggpps also shows a higher vulnerability to herbivores in the field. High concentrations of HGL-DTGs effectively defend valuable tissue against herbivores [1]. As suggested by Poreddy et al. [2], we made use of the genetically silenced lines and screened the excretion of M. sexta for detoxification products. Besides the expected glucopyranosyl-17-hydroxygeranyllinalool (RGHGL), we found several novel formed HGL-DTG-like compounds with different decorations (for example caffeoyl moieties) and further deglycosylation products. Here, we present a detailed MS-based analysis in frass of M. sexta and discuss the detoxification and the impact of this complex metabolic pathway to herbivory. References: [1]Heiling, S.; Plant Cell 22 (2010), 273-292. [2]Poreddy, S.; et al. in revision Utilizing mass spectral plant secondary metabolite profiles to unravel detoxification strategies and mechanisms in insects.

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The stable-isotope molecular oxygen enables pathway specific metabolome analysis using mass spectrometry

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Stable-isotopes such as 13C, 15N, and 34S have been used in metabolite annotation to trace a metabolite in fluxomics or to curate the elemental composition in a mass spectrometry (MS)-based targeted and untargeted analysis. Although oxygen is one of major elements in natural compounds, the stable-isotope oxygen (18O) has not been used effectively. Because oxygen atoms are derived from CO2, H2O, or O2 by each responsible enzyme, investigation of the origin of the oxygen atoms has a potential to suggest the presence of biosynthetic enzymes and to fill the gap between the metabolite and genetic information for further gene mining. Here, we investigate the 18O atoms in a flavonoid, as example, in the untargeted metabolome analysis of 18O2-labeled Medicago truncatula. M. truncatula, Jemalong A17, was grown and labeled in a sealed glass bottles which we developed for the economical 18O2-labeling of whole plants for four weeks. Each stable-isotope was fed from 13CO2, 15NH415NO3, K15NO3, Mg34SO4, 18O2, respectively. The 13CO2 and 18O2-air was exchanged once a day. The metabolites were extracted with 80% methanol and then analyzed by liquid chromatography (LC)-Orbitrap XL. The 18O2-labeled peaks were selected by ShiftedIonsFinder and the estimation of elemental composition and database search were performed by PowerGet. Additionally, unknown flavonoid candidates were searched by ShiftedIonsFinder and then one of them was identified by ultra-high performance LC-MS-solid phase extraction-nuclear magnetic resonance (UPLC-MS-SPE-NMR). In parallel, the multiple-stage MS analysis (MSn) for the candidate was performed by LC-Orbitrap Fusion. Because the 18O2-air is so expensive to fill the growth chamber, we developed the sealed glass bottle

system for economical 18O2-labeling. We succeed in labelling whole M. truncatula seedlings with 13C, 15N, 18O, and 34S using the system under same condition. After analysis by LC-Orbitrap XL, 3417 peaks and 3518 peaks were detected from unlabeled shoots and roots, respectively. For further annotation steps, 511 peaks and 353 peaks which have corresponding 18O2-labeled peaks and also have MS2 information were selected from each sample. According to the number of stable isotopes incorporated in the metabolite as an index, the elemental composition of 311 peaks (60.9% of 511 peaks) and 222 peaks (62.9% of 353 peaks) were estimated as single candidate within 3 ppm accuracy. Database search indicated that over one hundred peaks were identified as secondary metabolites, such as saponins and phenylpropanoids. Additionally, many unknown peaks were listed. We next searched flavonoid candidates among the unknown peaks using ShiftedIonsFinder. This tool calculates the hypothetical m/z values by adding a modification group to aglycone like a building block in silico. The resulting unknown peak at m/z 769.1624 was estimated as [[[apigenin] + 2 glucuronic acids] + coumaroyl group] by MSn analysis by LC-Orbitrap Fusion, and was further identified as apigenin 4'-O-[2'-O-coumaroyl-glucuronopyranosyl-(1-2)-O-glucuronopyranoside] by UPLC-MS-SPE-NMR. This metabolite incorporated four 18O atoms as found by comparing the full scan of unlabeled sample with that of 18O2-labeled sample. Among the isotopic ions, the ion having two 18O atoms showed highest intensity. Finally, we succeeded to find that one 18O atom was incorporated in 4'-hydroxy group of apigenin, the other one was incorporated in coumaroyl group by MSn analysis. This result might reflect that the function of cinnamic acid 4-hydroxylase (C4H) in flavonoid biosynthetic pathway. This study presented the basis of 18O-metabolome analysis and the potential of stable-isotopes to link metabolite annotation with gene mining.

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A Novel Strategy to Screen and Profile Steviol Glycosides of Natural Sweeteners in Food Using Microfluidic UPLC Ion Mobility

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Stevia rebaudiana Bertoni is a perennial shrub of the Asteraceae (Composite) family native to regions of South America. It is of significant economic value due to its high content of natural, dietetically valuable sweeteners in its leaves. It is referred to as "the sweet herb of Paraguay". Currently, stevia plant or extracts are used as sweeteners in South/North America, Asia and some European countries. The Joint FAO/WHO Expert Committee on Food Additives (JECFA) established regulations for steviol glycosides demanding a purity level of at least 95% of the seven chemically defined steviol glycosides. Here we present a unique approach to screen food products for steviol glycosides, using microfluidic chromatography and ion mobility, which provides unequivocal specificity and sensitivity. Extract of chocolate spread spiked with rebaudioside A to F, rubusodside, steviol, dulcoside A, steviolbioside and stevioside standards were analysed using microfluidic chromatography. Analysis was performed using a prototype post column addition (PCA) microfluidic device.The chromatographic gradient was provided from a ACQUITY® UPLC M-Class system with all separations occurring on the integrated microfluidic device. A 17 minute water (0.1% Formic Acid)/acetonitrile (0.1% Formic Acid) gradient at 2 µl/min was performed, make up flow 1µl/min IPA and injection volume of 5µl. Negative ionisation ion mobility data acquisition was performed. In this feasibility study unique sensitivity and selectivity in screening steviol glycosides complex mixtures has been achieved, where collision cross sections (CCS), accurate mass, fragment ions and retention time have been obtained to profile the steviol glycosides rebaudioside A to F, rubusodside, steviol, dulcoside A, steviolbioside and stevioside. Collision cross section measurements were readily obtained for the marker standards at 100fg/mL, and this information was used to create a scientific library incorporating the expected steviol glycoside CCS values. The results obtained clearly show the benefits of using the collision cross section measurements and ionKey MS with ion mobility. From the standards characteristic CCS assignment for glycosides isomer pairs (rubusodside 241.31 Å2 and steviolbioside 235.78 Å2),(rebaudioside B 261.19 Å2 and stevioside 269.64 Å2) (rebaudioside A 298.9 Å2 and rebaudioside E 289.2 Å2) have been determined. The approach taken, reduces the quantity of both expensive commodities i.e. high purity standards and solvent consumption, it has the potential to

negate the requirement to repeatedly purchase expensive high purity standards, for future screening assays. A chocolate spread extract was spiked with the analytes and routinely analysed using microfluidic chromatography combined with ion mobility, then screened routinely against the generated CCS library. When comparing the expected and measured collision cross sections, the CCS measurement errors were typically <0.4%. In addition, it has been possible to acquire the cleaned up fragmentation spectra, which are mobility resolved from co-eluting components. For the first time unique collision cross section measurements, precursor ion and corresponding isomer fragmentation spectra have been obtained using microfluidic chromatography CCS ion mobility screening for steviol glycosides. Enhanced sensitivity performance for ion mobility profiling in combination with microfluidic chromatography using a prototype microfluidic device.

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Targeted metabolic profiling using high-resolution accurate mass database to identify and confirm potential biomarkers in rose and sunflower plant extracts

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High resolution, accurate mass spectrometers are desired for both global and targeted metabolomics as they provide a high degree of selectivity over nominal mass systems. Unbiased targeted profiling using QTOF instruments allows for the collection of MS and MSMS data in a single-injection. This discovery data can be searched with a targeted list of metabolites, from many chemical classes, pathways and species. Metabolite identification with a high resolution accurate mass MS/MS library ensures increased confidence in assignment and purity scores of unknowns. Here we use a recently developed metabolite accurate mass MS/MS library and a single injection workflow to study plant extracts from different lots of rose petal and sunflowers and obtain quantitative data with qualitative confirmation. Extractions of rose petals from 3 different lots and an extraction of sunflower leaves were provided by Dr. Pfannstiel, U Hohenheim, Stuttgart. All extracts were combined into another “pooled” lot as a control. Mass Spectrometer: SCIEX TripleTOF® 6600 System. Data were acquired in both positive and negative ion mode using both SWATH(TM) acquisition and TOF-IDA experiments for comparison of the MSMS quality of spectra. HPLC gradient method used an Agilent 1290 (pump, column oven, autosampler). Column: Phenomenex Kinetex XB-C18 2.6µm, 2.1 × 100mm. Flow rate: 300 µL/min. Injection volume: 5 µL. Oven temperature: 40C. Mobile phase: A: H2O with 5mM NH4OAc. B: Acetonitrile 5mM NH4OAc. Multivariate Statistical Analysis (PCA) of the different lots produced unique differences (Figure 1) between the groups of samples. Importing and processing the data sets into MasterView(TM) software with the accurate mass metabolite spectral library confirmed the presence and relative amounts (in relation to other groups) of flavonoids catechin, quercetin, kaempherol and phloretin as well as several endogenous organic acids. The SWATH acquisition sets highlighted improved coverage of fragmented precursor masses. Quality of the deconvoluted SWATH acquisition MS/MS spectra is comparable or better than IDA-acquired product spectra providing more data points across the XIC chromatograms. 4Metabolite accurate mass library developed by Gerard Hopfgartner, Emmanuel Varesio, Tobias Bruderer, U Geneva, Switzerland. Accurate mass metabolite spectral library used in identifying potential biomarkers. SWATH acquisition highlighted increased metabolite coverage of all precursor masses.

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Changes in the metabolome of Eucalyptus due to variation of temperature and CO2 concentration

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Eucalyptus is the largest source of cellulose used in the paper industry in the world. Due to its rapid growth and high cellulosic yield, the plantation areas of eucalyptus have increased significantly. Due to anthropogenic activity there has been an increase in the emission of several gases in the atmosphere, particularly CO2. As a consequence, the global temperature is increasing. Plants respond to several environmental, and it is expected that higher CO2 concentration and temperature may cause drastic changes in physiological processes. To assess the effect of raising CO2 and temperature in the metabolism of eucalyptus, two species were grown at three temperature ranges and in each one CO2 was set to two concentrations. Seedlings of E. globulus and E. grandis were grown at 10°C, 20°C or 30°C, combined with two concentrations of CO2 (380 and 720 ppm), kept under these conditions for 6 weeks and sampled leaves were stored at -80°C. Before use, samples were extracted according De Vos et al. (2007), with modification. The extraction was optimized using a Qsonica Q700 sonicator. Thus both extracting methods were compared: regular and Qsonica Q700 ultrasonic bath. To evaluate the metabolic profiling, a 7.2T LTQ FT Ultrahigh MS equipped with a chiPoster based direct infusion nanoelectrospray ionization source, operating in both positive/negative ion mode was employed. Data acquisition was performed along the m/z 50-1000 range. The statistical comparisons were built based on HCA and PCA analyses. The optimized extracting procedure was faster and the number of metabolites extracted was higher than the normal method that uses an ordinary ultrasonic bath. The fingerprinting profile of eucalyptus samples was acquired using an Ion Trap (IT) analyzer followed by identification of the most intense ions by FT-ICR with electrospray ionization using the positive ion mode. Samples grown at 720 ppm CO2 appeared to be different in both E. grandis and E. globulus, clearly indicating alterations of metabolome. Differences between both extracting methods were also detected using ultrahigh mass spectrometry tools. The effect of the temperature variation was also observed in the eucalyptus samples. Interaction between temperature and CO2 concentration suggest the existence of a complex network of interactions, which in a long term may affect the growth rate of eucalyptus. The variation of temperature and CO2 levels modifies the metabolic profile of eucalyptus.

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Metabolic profiling in the grape ‘Campbell Early’ leaf tissue infected brown spot disease caused by Pseudocercospora vitis

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Brown leaf spot is major disease in the Korea vineyard, because grape ‘Campbell early’ planted vineyard about 70% as table grape but which is very susceptible cultivar to brown leaf spot. Pseudocercospora vitis is a pathogen, occur brown spot disease in grape leaf, which have close relation to Cercospora spp, secreted as a photosensitizing toxin during invade plant tissue. Many phytochemicals induced to protect in the plant tissue while pathogen attack. In grapevine, stilbene families were known phytoalexin related to disease resistance such as downy mildew, powdery mildew, and Botrytis rot. In most of case, phytoalexin was multiply response to various types of stress. Recently, many metabolomic approaches are revealed to possibility to find specific metabolites against specific pathogen. Brown spot leaf samples coused by Pseudocercospora vitis randomly collected ‘Campbell Early’ grapevine in harvest season. Leaf tissues were prepared with 5 mm punch for make a leaf disk at four different parts, 1: center of lesion, 2: lesion and border, 3: border of lesion, and 4: uninfected tissue. Collected leaf disks were grinded in liquid nitrogen and extracted with methanol:chloroform:water (5:2:2), extraction replicated five times. Each

extract was analyzed with GC-MS (Agilent 5980) after MSTFA derivatization. Raw files were trimmed with AMDIS program and searched with metabolomics Fiehn library. Mutivariate analysis is use Simca-p (V.12.0) program and Metaboanalyst (V.3.0) Four different collected tissues were grouped to the three groups (tissue 1, tissue 2, and tissue 3, 4) in the PCA score plot. Because tissue 3, 4 were similar metabolite variation, did not separate in the PCA plot. Total 296 metabolites analyzed through cut off ($p<0.05$) to find significant metabolite between tissues after ANOVA test, and then total 62 metabolites were significantly related to tissue differences. Most of metabolites (40), as caffeic acid, succinic acid, citric acid, were reduced from uninfected tissue to lesion. Oppositely, stearic acid, palmitic acid, and xylitol were increase in the close lesion tissue. Resveratrol and epi-catechin were increased tissue 2, 3, but those phytochemicals generally induced in the grapevine tissue against abiotic and biotic stresses. Especially mucic acid and arbutin were can find lesion and border tissue 2 which considered to typically related defense mechanism against the brown leaf spot. Cercospora spp, relatives of P.vitis, secreted a toxin during the disease development. Toxin generated singlet oxygen to broken plant cell wall. In this result, arbutin suspected to disease resistance because arbutin also related chemical to ROS reaction. Arbutin is a expected by metabolite profiling, species specific phytochemical against a photosensitizing toxin produce pathogen in grapevine

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Shifts in defensive metabolites in expanding versus mature leaves in Inga, a genus of tropical trees

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Plants use a variety of defensive strategies to reduce the impacts of herbivory. In tropical forest systems, where the rates of herbivory are high, foliar chemical compounds (secondary metabolites) are a prevalent defense. Relative to mature leaves, young (expanding) leaves of tropical plants are under high selective pressure from insect herbivores, due to their high nutritional content and low lignification levels (low morphological defense). As the majority of leaf damage occurs during foliar expansion, and damage is carried throughout the life of the leaf, expanding leaves are hypothesized to invest more in chemical defenses than mature leaves. We posit that expanding leaves will have higher relative concentrations and a greater degree of individual variation in chemical defenses than mature leaves. Field collections were conducted in Tiputini, Ecuador from multiple species of the Neotropical plant genus Inga. Developmental, morphological and chemical defense traits were initially measured and used to select six species of Inga to examine chemical defense differences between expanding and mature leaves. Leaf samples (n=5 /leaf developmental stage/species) were dried, ground, extracted in an acetate/acetonitrile buffer and analyzed using UPLC-MS on an Acquity UPLC®I-class/Xevo® G2 Q-ToF on a 1.7 µm, 2.1 x 50 mm Acquity BEH C18 column (Waters Corporation, USA). A butanol-HCl assay was additionally performed to test the degree of cell wall-bound metabolites. Raw data was annotated using Bioconductor v3.0 and XCMS in R (v3.1.0, The R Project for Statistical Computing) and analyzed using MetaboAnalyst v3.0. Preliminary findings indicate that the chemical defense profiles of expanding and mature leaves differ within each of the Inga species analyzed. Unsupervised Principal Component Analysis (PCA) demonstrates clear chemical differences in the expanding and mature leaf metabolomes. Supervised Partial least squares Discriminant Analysis (PLS-DA) demonstrates significantly different chemical defense metabolomes for within- and between-species comparisons of expanding and mature leaves. The liquid extraction method yielded a higher proportion of secondary metabolites extracted and total ion current (TIC) values for expanding leaf samples. Venn diagrams demonstrate a much greater variation in leaf chemical defenses in expanding than mature leaves. This result indicates that there is less chemical defense overlap in expanding leaves, which may facilitate escape from specialist herbivores adapted to a specific chemical defense metabolome. Mature leaves do not appear to have a higher degree of cell wall-bound secondary metabolites, although ongoing analyses may reveal the degree to which foliar chemical defenses shift in expanding versus mature leaves. Metabolomic profiling of foliar chemical defenses highlights the importance of vulnerable leaf expansion stages to plant survival in tropical forests.

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Short oligogalacturonides induce defense responses in Arabidopsis thaliana

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In an environment full of potentially harmful microbes, efficient sensing of danger and rapid triggering of defenses are crucial for the survival of plants. Besides the recognition of pathogen associated compounds plant immunity relies on the ability to sense endogenous molecular patterns that are present only when plant tissue is infected or damaged. Fragments of plant cell wall released as a result of microbial enzyme action or wounding can act as such damage-associated molecular patterns (DAMP) that efficiently trigger defenses. Major end-products from the degradation of pectin by plant cell wall degrading enzymes (PCWDE) are oligogalacturonides (OGs) with varying degrees of polymerization (DP). We have characterized the accumulation of various phytohormones in response to short (DP < 10) OGs. Phytohormones (salicylic acid (SA), jasmonic acid (JA), abscisic acid (ABA) and auxin (IAA)) were extracted from frozen and ground plant material and analyzed with Waters Acquity UPLC-ESI/QTOF/MS (Waters, Milford MA, USA) in negative sensitivity ion mode. Mass range was 50-600. The compounds were separated on a Acquity UPLC® BEH C18 column (1.7 µm, 50 x 2.1 mm, Waters, Ireland) in 40°C. The mobile phase consisted of (A) H2O and (B) acetonitrile both containing 0.1% HCOOH. A linear gradient used started with 95 % of A and changed to 5 % in 14 min, then back to 95 % in 14.1 min and left to equilibrate for 1 min. The injection volume was 2 µl and flow-rate was 0.6 ml/min. Previous studies have characterized the effects of long OGs (DP>10) on the transcriptome level in Arabidopsis during early stages of defense signaling. The role of short OGs (DP<10) in triggering plant defense in transcriptome or metabolome level has not been well characterized. To explore the global impact of short OGs in modifying the plant transcriptome and biosynthetic routes of phytohormones, we designed an assay comparable to the study of long OGs. To this aim, plant seedlings were grown in vitro as previously published and treated with two different types of short OGs: commercial trimers or short OG-mix, or with plain mock (_ MS). Samples for RNA sequencing were collected 3 h and for hormone analysis 0, 3, 7 and 24h post treatment, respectively. The resulting comparative transcriptomic data from this work reveals that trimers and the short OG-mix induce the expression of many defense-related genes in Arabidopsis. Several genes associated with hormonal response pathways (ET, JA, SA) were mainly uPoster regulated. Additionally, the plant treatments with short OGs led to down-regulation of genes promoting plant growth and development i.e. gene sets involved in photosynthesis, general metabolism, development and transcription were down-regulated. UPLC-MS data also supports the previous findings that short OGs induce biochemical pathways of the phytohormones and hormone accumulation in Arabidopsis. Various phytohormones in Arabidopsis are accumulated in response to DAMPs and are induced by short OGs.

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Screening the candidate genes related to the tobacco pigments regulation by correlation analysis

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Tobacco plastid pigments, which exist in plant cell organelles (including chloroplasts and chromoplasts),

are divided into two broad categories: chlorophyll and carotenoid, including chlorophyll a, chlorophyll b, neoxanthin, lutein and beta-carotene. As we know, pigments play a very important role in the process of plant photosynthesis: participating in light absorption and preventing the photooxidation of precursor cells. Meanwhile, carotenoids are signal molecular precursor when plant response to external stimulations. Recently, it was confirmed that carotenoids functioned in the morphogenesis of light, the non-photochemical inhibition reaction and lipid peroxide reaction, the synthesis of hormones, and attracting pollinators, etc. The tobacco leaves were immediately frozen by liquid nitrogen after collection for transcriptome analysis. Transcriptom analysis was carried by
using Nicotiana tabacum WT (Whole Transcriptome) gene chips, which
containing 81491 genes, each experiment has three replicates. For pigments concentration detection, the frozen leaves were freeze-dried and grounded into tobacco leaf dry powder. HPLC method was used to detect the concentration of five pigments (chlorophyll a, chlorophyll b, neoxanthin, lutein and beta-carotene) in tobacco leaves.Then, the tobacco leaf dry powder was extracted by 95% acetone under 20 min ultrasonic treatment. After a filtration, the sample then could be detected. The correlation is analyzed by Pearson product-moment correlation coefficient. To investigate the genes related to pigments metabolism, tobacco leaf samples obtained from 22 different areas (each area has six biological reduplication) in China were emploied to research the correlation between pigment content and 81491 genes expression quantity. These samples covered 9 provinces of China, including nine cultivars (K326, Zhongyan 100, Yunyan 87, Yunyan 105, Yunyan 97, Yuyan 10, Nanjiang 3, Bina 1 and Guiyan 2). The Pearson correlation coefficient between 81491 genes expression quantity and each pigment were respectively calculated, and then the top ten genes of positive correlation and negative correlation were picked up. All of these correlation coefficients were bigger than 0.7, which indicated that the correlation of these genes and pigments was good. From the results, it can be seen that 13 genes were related to both chlorophyll a and chlorophyll b, including 498650, 522233, 498649, 526679, 508945, 492608, 492239, 530750, 492240, 495922, 495921, 495919 and 495920 gene. Three genes were associated with neoxanthin, lutein and beta-carotene, including 495922, 495919 and 495920 gene. There were three genes associated with all five pigments including 495922, 495919 and 495920 gene. All these three genes were positive correlation with five pigments. To sum up, by correlation analysis between pigments concentration and transcriptomes, some genes have high correlations with pigments were supposed to be candidate genes for pigments regulating. And further experiments would be done to verify whether these genes regulate the pigments metabolism. Candidate genes for a particular substanceregulating could be screened by associating transcriptome data with metabolite data such as pigment.

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Correlation analysis promoting the discoveries of new candidate genes regulating the sterols metabolism in tobacco

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Sterols are essential molecules for all eukaryotic organisms. Tobacco sterols, mainly including: stigmasterol, β-sitosterol, cholesterol, brassicasterol and campesterol, are present in tobacco smoke, and appear in plasma of mammals exposed to cigarette smoke. Tobacco sterols may be important in the pathogenesis of smoking-induced lung and vascular diseases. Sterol concentration in tobacco and its regulator were a concerned question, which attracts many scientists to study it. Although several plant synthesis enzymes were cloned, but the exactly mechanism of sterols synthesis was unclearly. In this study, correlation analysis between metabolites and transcriptomes was used to investigate the candidate

genes possibly involved in the regulation of sterol metabolism. Fresh tobacco leaves were quickly frozen,crushed in liquid nitrogen and stored in a -80 °C freezer before transcriptomeanalysis. Frozen tobacco leaves were lyophilized in a freeze-dryer and grounded into powder. For sterols detection, five kinds of sterols were determined from tobacco leaves by GC-MS/MS. A Plant RNA Extraction Kit (Gene Answer) was used to extract total RNA, and DNaseIwas used to remove any DNA contamination from the RNA samples. Agarose gel electrophoresis was used to test RNA integrity, and a mini-spectrophotometer (NanoDrop 2000) was used to test the purity and concentration of the RNA. Transcriptome analysis was carried by using Nicotiana tabacum WT (Whole Transcriptome) gene chips, which including 81491 genes, each experiment has three replicates'. Nine cultivars were used including K326, Zhongyan 100, Yunyan 87, Yunyan 105, Yunyan 97, Yuyan 10, Nanjiang 3, Bina 1 and Guiyan 2, which were cultivated in 22 locations spreading in the North of China, area of Yangtze River and South of China respectively, and each location has 6 biological duplications. Five main sterols including stigmasterol, β-sitosterol, cholesterol, brassicasterol and campesterol were determined. Transcriptomic data were obtained by using Nicotiana tabacum WT gene chips. The correlation between the level of gene expression and sterols contents were analyzed by calculating the Pearson product-moment correlation coefficients. Then ten of the most correlative genes (positive correlation and negative correlation) were listed respectively. There were 4 genes related with more than two sterols. x_521244 was negative correlated with cholesterol and stigmasterol (correlation coefficients -0.669 and -0.661), x_532297 was negative correlated with campesterol and stigmasterol (correlation coefficients were -0.583 and -0.652), x_550706 was negative correlated with campesterol and stigmasterol (correlation coefficients were -0.600 and -0.637), x_484934 was negative correlated with cholesterol and stigmasterol (correlation coefficients were -0.675 and -0.614). It is interesting that all these four genes were negative correlated with stigmasterol. In further study, more experiment will carry out to confirm whether these genes regulate the sterols metabolism. Correlation analysis between metabolites and transcriptomics could be an efficient method to screen new candidate genes related to the metabolism.

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Landscape of the lipidome and transcriptome under heat stress in Arabidopsis thaliana

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Environmental stress causes membrane damage in plants. Lipid studies are required to understand the adaptation of plants to climate change. Here, LC-MS-based lipidomic and microarray transcriptome analyses were carried out to elucidate the effect of short-term heat stress on the Arabidopsis thaliana leaf membrane. Vegetative plants were subjected to high temperatures at 30, 34 and 38 degrees C for one day, and then grown under normal conditions at 22 degrees C for 2 days. Sixty-six detected glycerolipid species were classified according to patterns of compositional change by Pearson's correlation coefficient. Triacylglycerols, 36:4- and 36:5-monogalactosyldiacylglycerol, 34:1-, 36:1- and 36:6-phosphatidylcholine and 34:1-phosphatidylethanolamine increased by the stress and immediately decreased during recovery. The relative amount of one triacylglycerol species (54:9) containing alpha-linolenic acid (18:3) increased under heat stress. These results suggest that heat stress in Arabidopsis leaves induces an increase in triacylglycerol levels, which functions as an intermediate of lipid turnover, and results in a decrease in membrane polyunsaturated fatty acids. Microarray data revealed candidate genes responsible for the observed metabolic changes. Heat stress induced decrease in unsaturation levels of membrane glycerolipids in leaves; triacylglycerol accumulation may be involved in this process.

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Lipidomic analysis of Arabidopsis thaliana revealed a metabolism of glucuronosyldiacylglycerol that is essential for plant growth under phosphorus deprivation

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Phosphorus (P) is an essential macronutrient for plant growth. Former researches have revealed the importance of remodeling of membrane lipids in plants under P-limited conditions. Under P deprivation, phospholipids significantly decrease and non-phosphorus glycoglycerolipids including sulfoquinovosyldiacylglycerol (SQDG) and galactolipids increase. Recently, we identified a novel plant glycolipid glucuronosyldiacylglycerol (GlcADG) from Arabidopsis, rice, soybean, and tomato grown under P-limited conditions. Biosynthesis of GlcADG requires SQDG synthase (SQD2), and the GlcADG-deficiency in sqd2 mutants led to the severe growth defects under P depletion (Okazaki et al., Nat. Commun. (2013)), indicating the important function of this lipid class. In this presentation, we will report the result of the detailed lipidome analysis of Arabidopsis, and the discovery of new metabolites related to GlcADG. Arabidopsis plants (Col-0, ugp3, sqd1, and sqd2) were grown on agar-solidified Murashige and Skoog medium at 22°C. After a 14-d-incubation, the plants were transferred to agar plates containing either P-sufficient and P-deficient medium based on the sterile Arabidopsis medium (Härtel et al., PNAS (2000)). After another 14-d-incubation, plants were harvested and stored at −80°C. Crude lipid extracts were prepared as reported previously (Okazaki et al., Physiol. Plant. (2015)). Briefly, the frozen plant material was crushed into the fine powder, to which a mixture of methyl tert-butyl ether : MeOH (3:1) was added. The hydrophilic metabolites were removed from this fraction by washing with water. The resulting organic layer was concentrated, reconstituted in EtOH, and subjected to LC-MS analysis. Crude lipid fractions extracted from shoots of Arabidopsis thaliana grown under phosphate-sufficient and phosphate-deficient growth medium were analyzed on a reversed phase HPLC coupled with ESI-qTOF MS to detect the stress-inducible changes in lipids such as glycerolipid (phospholipids, glycoglycerolipids), sphingolipids (ceramides, glycosylceramides), and sterol derivatives (sterols, sterylglycosides, acyl sterylglucosides). Comparison of the dataset indicated that significant decreases of all the detected phospholipids and complementary increases of non-phosphorus glycoglycerolipids such as SQDG and digalactosyldiacylglycerol, and GlcADG. In addition, we found an induction of accumulation of unknown metabolites in P-starved plants. These unknowns also could be detected from root tissues, and their levels were elevated under P deprivation as observed in shoots. MS/MS of these unknown metabolites suggested that they were acylated derivatives of GlcADG and they are biosynthesized by the function of SQDG synthase (SQD2) like GlcADG. Investigation of a series of SQDG-deficient mutants of Arabidopsis revealed that only sqd2 mutant lacks both GlcADG and these unknown lipids. These data suggest that GlcADG is further metabolized into other lipid species discover in the present study. Presumable derivatives of GlcADG that is essential for phosphorus-starved plant growth was discovered from Arabidopsis by lipidomic analysis.

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Metabolite Profiling of the Leaves and Stems of Lespedeza maximowiczii During the Growth Period and Correlation with Bioactivities

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Lespedeza species have known as traditional herbal medicines for treatment of diarrhea and diabetes. Many researchers have reported several secondary metabolites isolated from Lespedeza sp. and their bioactivities such as anti-oxidant, anti-melanogenesis, and anti-diabetic effects. So far, L. maximowiczii, one of Lespedeza sp., has not sufficiently been reported. Furthermore, the explanation for primary and secondary metabolites from each part of this plant is inadequate yet. In this study, we aimed to examine discriminated metabolites according to growth period in two different parts of L. maximowiczii through metabolite profiling and to understand the correlation between bioactivities and these metabolites. In this study, the leaves and stems from L. maximowiczii were collected by harvesting at 3, 4, 6, 8, 15 and 18 months after germination. We investigated the metabolites discriminated from the each growth period in leaves and stems from L.maximowiczii by using gas chromatography (GC)-time of flight (TOF)-mass spectrometry (MS) and ultra-performance liquid chromatography (UPLC)- quadrupole-time of flight (Q-TOF)-MS with multivariate analysis including principal component analysis (PCA) and partial least

squares discriminant analysis (PLS-DA). We also tested the antioxidant and tyrosinase inhibitory activities. Correlation map were used to show relationship between bioactivities and metabolites. The leaves and stems of L. maximowiczii were clearly separated according to growth period in PCA score plots obtained from GC-TOF-MS and UPLC-Q-TOF-MS analyses, respectively. Especially, both leaves and stems harvested at 6 months after germination were significantly distinguished from those at other months. Among discriminated variables according to the growth period from GC-TOF-MS data, most metabolites showed relatively higher or lower contents at 6 month after germination than other months. Of them, these metabolites containing lower amounts, such as GABA, xylose, and galactose were more detected in leaves, while those showing higher contents, including aspartic acid, valine, isoleucine, tyrosine, threonine, serine and malonic acid, were more determined in stems, except for some metabolites having content differences in common. Moreover, both the leaves and stems (at 8 month) harvested in November showed the largest contents of sugars including fructose, glucose and sucrose which have known to increase in response to cold stress. In UPLC-Q-TOF-MS analysis, the leaves and stems of L. maximowiczii at 6 month also contained higher levels of most flavonoids, such as isoflavone, flavonone, flavonol, flavone-glucosides, flavonol-glucosides, which were identified as distinguished metabolites depending on growth period. In particular, luteolin 6-C-glucosyl-8-C-arabinoside and luteolin-O-glucoside, with the highest levels at 8 months which were related with cold stress, were detected in leaves. In stems, lespedol C, lespedezol A6 and lespeflorin B4 showed the largest contents, while apigenin-6-C-glucosyl-8-C-arabinoside, isoorientin-2-O-gluside and kaempferol showed the lowest contents at 3 months after germination. Together, both the leaves and stems at 6 months after germination possessed the highest tyrosinase inhibitory activity indicating that daidzein, isovitexin, desmodianone H, and uncinanone B contributed to high positive correlation. From these results, we revealed that L.maximowiczii underwent the biggest metabolites changes at 6 months after germination in both leaves and stems and it also affected its activities. It was the first study on metabolite differences and activities concerning plant growth in L. maximowiczii using MS-based metabolomics.

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Use of HCA heat map of red propolis to design experiments based on complex natural products

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Natural products are complex matrix to work with, considering that secondary metabolites in plants comprise a wide variety of compounds. Making choices on what experiments perform or where to begin working with a natural product might be tricky. Preliminary experiments and multivariate analysis of the data can help to visualize the results and set the parameters for future experiments. Red propolis is a bee product with promising therapeutic activities, fact that increases the interest in knowing its chemical composition. Bees collect this resin from different buds and branches from different plant sources. Thus to determine its composition it is necessary to isolate the constituents of red propolis, but the interest of the resin lies in the bioactive constituents. Data of relative abundance of the ions present in red propolis obtained from preliminary analysis by UHPLC-MS and data of antioxidant and antimicrobial activities of raw red propolis collected in different regions of Brazil were used to perform the analysis. Data were normalized and scaled, and a Pearson's correlation ($p \leq 0,05$) along with a hierarchical clustering analysis (HCA) was performed to obtain a HCA heat map using MeV software. Two main results were observed in this metabolomic approach. Firstly the theoretical correlation between some of the ions with each of the biological activities was observed. Some ions were grouped together in the HCA tree with the antioxidant activity evaluated by ORAC and other ions were grouped with the antioxidant activity assessed by DPPH. These methods have different reaction pathways and thus it is expected that the metabolites responsible for the antioxidant activity are different depending on the assay performed. It was also possible to determine the ions that relate most with the antimicrobial activity. It was also possible to determine the main compounds in each sample. These two results together allow us to design future experiments by selecting the most important compounds to isolate from red propolis based on their correlation with the biological activities and determine the samples which they should be isolated from based on their abundance in each sample. HCA-heatmap is frequently used in gene expression analysis. Here we propose its use to design metabolomics experiments of natural products.

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Effect of Genetics and Environment on the Metabolome of Maize Hybrids Using GC/MS and LC/MS

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It is imperative to understand biological variation in expression of metabolites due to genotype, trait, environment, or both to assess the potential of metabolomics to supplement compositional analysis for substantial equivalence assessment of genetically modified crops. In the present study, we evaluated the biological variation of metabolites in various maize hybrids due to environment (E), genotype (G), or trait (T) effects, and different interactions (GxE, ExT, GxExT). A total of 480 forage and 480 grain samples from 21 genetically diverse non-GM Pioneer® brand maize hybrids grown at eight North America locations were analyzed using complementary analytical platforms GC/MS and LC/MS for a wide range of metabolites classes. A total of 166 and 137 metabolites were detected in forage and grain samples, respectively, by GC/MS, while 1341 and 635 metabolites were detected in forage and grain samples, respectively, by using LC/MS Univariate and multivariate analyses were utilized extensively to investigate environment, genotype, or trait effects, and interaction of the three on the metabolome profiles. In forage, the environment affected 36% to 84% of the metabolites, while less than 7% were affected by genotype. In grain, the environment affected 12% to 90% of the metabolites, whereas less than 27% were affected by genotype. Less than 10% and 11% of the metabolites were affected by traits in forage and grain, respectively. Additionally, the effects of drought and disease resistant traits were studied within each location, and less than 10% of the metabolites were affected by these traits. Unsupervised PCA and HCA analyses revealed similar trends, environmental effects were much stronger than genotype or trait effects. Overall, the results of this comprehensive study support and extend the previous findings about the environmental and genetic perturbations on the maize metabolome. Our findings demonstrate that the combination of GC/MS and LC/MS based metabolite profiling followed by comprehensive statistical analysis is a powerful approach to identify the relative impact of environmental, genetic and trait effects on the forage and grain composition of maize hybrids. Potential utility of metabolomics for substantial equivalence assessment for GM crops

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Computational and integrative omics methods to study metabolic phenotypes

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Analysis of metabolomics data in combination with other omics or metadata can provide vital insights on how genetic information and environmental factors can influence cellular metabolic responses and phenotypic characteristics. However, for large sample experiments performed in different batches, the biological differences between samples are often confounded with obscuring non-biological sources of variability (batch effects) leading to systematic errors. If these variations are not corrected, they lead to erroneous biological interpretations. In this study, we have developed (i) statistical methods to mitigate batch effects, while preserving the biological source of variation, and (ii) a data-dependent multi-omics strategy that combines metabolomics, transcriptomics and transcriptome-dependent in silico genomics to provide insights into the coordinate regulation of multiple components in the metabolic network. In an untargeted metabolite survey of 22 naturally varying algal strains, compared over two growth stages and run in four batches, we used significance tests and analysis of variance measures to evaluate the impact of batch effects on the metabolite features. We then developed a filtering procedure based on the singular value decomposition to remove batch effects from the metabolomics dataset. In another study, in order to uncover the effect of a single gene mutation on the Arabidopsis metabolic network, we performed sub-

pathway enrichment analysis using differential genes and metabolites. We then used a network-guided guilt-by-association approach and developed constraint-based promoter motif networks to uncover novel relationships between genes at a regulome level. Deriving meaningful biological information from complex systems requires robust statistical methods. Results from this study highlight the utility of using systems-level statistical approaches to uncover novel relationships among cellular processes. Firstly, given a set of linear combination of principal components estimated from the metabolomics data, we developed a filtering procedure that (i) preserves signals of demonstrable biological origin (strain-related variation in mass feature intensity) after minimizing the influence of batch effects, and (ii) does not require quality controls or standards for removing unwanted variation. We use the non-targeted algal metabolomics data to show that the proposed filtering procedure can be used to effectively remove nuisance variation caused by batch effects, while preserving strain-specific differences that can serve as a direct indicator of biochemical phenotype. This dataset was then analyzed in an integrative manner to derive associations between genotype, physiological measurements and metabolic divergence among the algal strains. Secondly, by utilizing the combined power derived by integrating genomic relationships and gene expression outcomes with metabolite profiling, we generated specific testable hypotheses to understand the relationships between multiple components of biological processes. This analysis helped discover coordinated regulation of metabolic pathways associated with stress response and secondary metabolism in Arabidopsis. Taken together, we show that multi-omics approaches can be used to understand (i) how organisms respond to perturbations by modulating their gene expression levels, and (ii) how metabolic network is reprogrammed through orchestration of coordinated gene-metabolite changes. Using a multi-omics approach, this study provides insights into coordinate regulation of metabolic network components in complex cellular phenotypes.

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A metabolomics approach to improving plant protection: 1H NMR and MS screening of pathogen-host responses in trees.

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Phytophthora is a genus of soil and air borne plant pathogens which live up to their Greek name of plant destroyers. New Zealand has several Phytophthora species that are impacting on the sustainability of iconic native trees, plantation forests, and apples. A fledgling research programme at Scion is taking a systems biology based approach to improve disease management and advance current knowledge of Phytophthora-tree interactions by the use of 1H Nuclear Magnetic Resonance (NMR) and Mass Spectroscopic techniques to investigate changes to the metabolomic profiles during the asymptomatic host-pathogen interaction phase of infection. To study the host-pathogen interactions Scion is combining a systems biology approach to a multi-host-multi-pathogen model. Three tree species; Pinus radiata, Kauri (Agathis australis) and a variety of apple trees (Malus domestica) will be challenged by eight Phytophthora species, respectively. NMR spectroscopy was used in an untargeted metabolomics approach to the needles of susceptible and resistant Pinus radiata cultivars challenged with Phytophthora under laboratory conditions. The metabolomic profiles were investigated at different stages of infection with NMR and further examined by mass spectrometric technologies to characterise chemicals present in infection states. These analyses are being directly compared to parallel transcriptomic and histological analysis to provide chemotypic and phenotypic data to inform tree breeding programmes underway for each host species. The initial research has focused on Pinus radiata challenged with Phytophthora pluvialis in a detached needle assay. Preliminary results of 1H NMR fingerprinting on the comparison between controls and infected needles have produced chemical changes that align with pathogen infection. This work will report initial results for the non-targeted metabolomic analysis of the P. radiata needles susceptible and resistant cultivars of P. radiata with 1H NMR fingerprinting. Development of a diagnostic tool to identify stands of trees under pathogen attack before outwards signs of disease are present.

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Differential Lettuce Roots Metabolome Response to Salt and Zinc Stress

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Although zinc occurs naturally, some human activities like mining, smelting, steel production, as well as burning coal can lead to high levels of contamination even in agricultural soils. In most cases, being zinc present as salt, it can exert osmotic stress toward plants. However, being this element a heavy metal, specific effects on plant metabolisms can be also forecast. In this work, lettuce has been used as a model to investigate and compare salt and zinc stresses at root metabolome level. Lettuce (Lactuca sativa L.) plants were grown in either zinc sulphate or osmolar sodium chloride soil, and roots were harvested after 30 days of stress induction. Samples were extracted in aqueous methanol and analyzed through UPLC chromatography coupled to quadrupole-time-of-flight mass spectrometry (UPLC-QTOF). Raw data were mined considering accurate mass together with isotope spacing and ratio, and then compounds were identified using the PlantCyc database. Compounds were aligned in Mass Profiler Professional, filtered by frequency within each treatment (only those compounds detected in 5 out of 5 replications were retained) and exported for re-identification in raw data. After this recursive identification, compounds were finally re-exported in Mass Profiler Professional for statistics and multivariate chemometrics. Although a significant decrease in the number of compounds has been observed, the recursive analysis resulted in a better defined dataset, as clearly evidenced by both unsupervised cluster analysis and principal component analysis. Aimed at pointing out differences at metabolome level and interpretate the results at physiological level, the most useful results were gained using the output from Volcano plot and partial least square discriminant analysis. The subsequent Volcano plot and Venn analysis were used to discriminate between the effects related to osmotic/oxidative stress (common with salinity). In general, differential metabolites were shared between salt and zinc stress, although quantitative differences could be noted. Regarding carbohydrates, fructans increase was more evident under saline conditions; the same trend was observed for starch degradation, while cellulose and hemicellulose degradation had opposite trend. Flavones increased more under salt stress, probably to cope with the secondary oxidative stress, while the increase in terpenes and alkaloids was higher in zinc-stressed roots. Lipids metabolism was also significantly affected by both stresses, and more by salinity, being fatty acids and phospholipids biosynthesis decreased and diglicerides lowered. Ascorbate degradation products and polyamine catabolites were increased in zinc-stressed roots, while production of homoglutathione (and hence phytochelatins biosynthesis) was induced by salt stress. Finally, some other specific compounds, reported to be linked to drought or salinity could be pointed out (e.g. serotonin and phytoanticipins), whereas the effect of the stresses on aminoacids metabolism was unclear and contrasting results were achieved for the different anabolic pathways. Recursive analysis was a powerful tool to improve dataset quality, hence discriminating the zinc-related specific effects on root metabolome.

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Determining the Roles of Walnut Phenolic Metabolism in Pellicle (Seed Coat) Pigmentation

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English Walnut (Juglans regia) is one of California's most significant agricultural commodities, with annual production valued at more than $1.3 billion. A key factor for walnut exports is pellicle (seed coat) color: pale nuts command higher prices in Europe; red nuts are favored in China. Recent studies have demonstrated that pellicle color is influenced by phenylpropanoids, a class of compounds that includes tannins, anthocyanins, melanins, lignins and monophenols; many of these compounds are pigmented. Pellicles isolated from cultivars presenting unique pellicle colors (pale, tan, red) were used to study phenylpropanoid biosynthesis and pathway regulation profiles during nut maturation. Of particular interest are the metabolites that control pellicle pigmentation and the biosynthetic pathways involved in phenylpropanoid biosynthesis in the pellicle. A non-targeted metabolomic analysis was performed by Metabolon (Durham, NC) on pellicle samples from three genotypes exhibiting distinctive pigmentation differences. Nuts collected from the cultivars Chandler - pale, Gayle's Caramel – tan, and Robert Livermore – red, were examined at three developmental stages. Data were normalized for internal

consistency by processing a constant weight per volume of extraction solvent for each sample. No post hoc mathematical normalization was imposed on the data. Data were scaled to the median value for each compound, and then missing values were imputed with the minimum detected value for that compound. Statistical calculations were performed using natural log-transformed scaled imputed data. Metabolites were mapped to their biochemical pathways using the MapMan program. A total of 209 metabolites were detected in the pellicle samples examined. As anticipated, extensive growth-specific changes in metabolism were observed in all genotypes. Fewer differences were evident between cultivars, when examined at a specific growth stage; most of the observed differences between the cultivars are differences in secondary metabolite content. One of the primary groups of metabolites to exhibit cultivar-specific differences is the phenylpropanoid pathway. The anthocyanin, cyanidin galactoside was found to be present only in the genotype Robert Livermore, at all stages of growth. This compound is most likely, primarily responsible for the distinctive red pigmentation of the RL pellicle. When Chandler (pale pellicle) was compared against Gayle's Caramel (tan) and Robert Livermore (red) higher levels of flavonoids were found to accumulate at all three developmental time points. These flavonoids include quercetin, quercetin 3-galactoside, quercetin 3-O-glucoside. Flavanol epicatechin and proanthocyanidin monomers were elevated in Robert Livermore relative to Chandler. Chlorogenate levels were higher in Gayle's Caramel and Robert Livermore than in Chandler and crytochlorogenate was higher in Robert Livermore than in Chandler. Meanwhile, the levels of quercitrin, procyanidi B1 and coumaryolquinate were high in Chandler than in Gayle's Caramel and Robert Livermore. Differences in these secondary metabolite contents may just reflect genotype-specific variation between the three cultivars. Alternatively, it is possible that these results reflect a role for various phenolic compounds in co-pigmentation of the walnut pellicle. In plants, pigmentation of non-photosynthetic tissue has been widely attributed to three classes of compounds; carotenoids, betalains and anthocyanins. It has been proposed that interaction with or co-accumulation of pigmented metabolites with other colorless phenylpropanoidsincluding flavonoids, may result in variation of the primary pigmentation attributed to a particular anthocyanin. This phenomenon has been described in the literature as "co-pigmentation." This is the first description, to our knowledge, of the metabolites present in different walnut cultivars presenting distinct pigmentation.

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Trans-omics analysis as a tool for understanding the complex physiological effects of arogenate dehydratase knockout in Arabidopsis thaliana.

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Phenylalanine derived metabolism produces a variety of chemicals of great to plants life. This biosynthetic pathway had a pivotal importance in plant evolution, being responsible for the successful adaptation to the terrestrial environment. Lignin is its main product and the second most abundant biopolymer in the planet reaching upwards of 30% of all carbon fixed through photosynthesis. Besides lignin, the phenylpropanoid pathway also leads to several small molecules that play different roles in plant physiology and in interactions with its environment. As part of a larger effort to engineer plants for improved use as biofuels, our group has produced several A. thaliana lines with single and multiple knockouts of arogenate dehydratase isoenzymes, resulting in significant reduction in lignin content. We performed an untargeted metabolomic profiling using ultra-performance liquid chromatography coupled with accurate quantitative time of flight mass spectrometry (UPLC-ESI-TOF) analysis of hydro-alcoholic extracts of leaves from wild type and several ADT knockout lines. Data generated was processed using XCMS and CAMERA followed by manual data analysis. In parallel, we have performed a comprehensive photosynthetic characterization of the plants using the phenomics facilities at our disposal The metabolomic profiles showed that ADT modulation effected several phenylalanine-derived metabolites, as expected, but also unveiled many unexpected pleiotropic effects. The unforeseen effects included reduced content of several glucosinolates, phenolics and apocarotenoids that could have impacts in on plant physiology and general fitness. Analysis of photosynthetic parameters also revealed significant

impacts that point to energetic unbalance caused by the removal of the phenylpropanoid metabolism, a significant energy sink. Combination of metabolomics and phenomics characterization provide great insight into plant complex response to ADT modulation in A. thaliana..

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Metabolome dynamics of crassulacean acid metabolism (CAM) in the facultative CAM species, Mesembryanthemum crystallinum

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Crassulacean acid metabolism (CAM) is an elaboration of C3 photosynthesis wherein carbon assimilation occurs at night resulting in reduced daytime water losses through a temporal separation of primary C4 and secondary C3 carboxylation reactions. Understanding the biochemical regulation of this reduced water lost in CAM plants is essential for developing novel strategies for improving crop plants to grow in water-limited environments. To unravel the underlying biochemical basis of CAM, comparative metabolomic profiling were performed on wild type (WT) and CAM-deficient mutant (MT) in the facultative CAM plant, Mesembryanthemum crystallinum, performing either C3 photosynthesis or CAM, induced by water-deficit stress treatment. Leaves were collected every 4 h over a 72 h time course under both 24 h light/dark and 48 h light/light conditions to characterize the circadian clock-controlled metabolome in both the C3 and CAM states. Under water-deficit conditions that induce CAM, greater numbers of metabolites become rhythmic indicating that the stress-adaptive and CAM biochemical machinery is directly under circadian clock control. Comparative analysis of the two metabolomes identified key metabolites underlying the biochemical regulation of CAM in both WT and MT ice plants. Most C4 and tricarboxylic acid (TCA) cycle metabolites exhibited circadian clock-controlled increases in WT CAM-performing plants, whereas the CAM-deficient mutant accumulated far fewer of these rhythmic compounds. The CAM-deficient mutant failed to accumulate starch breakdown products, such as maltose and maltotriose, but showed increased accumulation of soluble sugars, sugar alcohols, nitrogen-rich compounds, and lipids providing novel insights into the metabolic flux requirements for CAM performance. Together these results provide an unprecedented view into the complex biochemical regulatory events orchestrating CAM.

poster 559

Regulation of pathway activity and metabolic flux in oilseeds and cereals

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The biosynthesis of storage lipids, proteins and starch in crop seeds has been studied extensively, and yet our understanding of the nature of the coordinating components is still fragmentary. We particularly aim to discover regulatory motifs for flux control in central plant metabolism. To elucidate regulatory motifs in plant metabolism, we apply targeted LC/MS approaches covering ~100 metabolic intermediates including phosphorylated sugars (glycolysis), organic acids (citric acid cycle), nucleotides and their sugars, free amino acids and soluble sugars. Such analysis is combined with 13C-isotope tracer studies for flux analysis, proteomics and transcriptomics. Current work on cereals and oilseeds indicated that (1) there is a close coordination between energy metabolism and carbon partitioning pathways with increased demands for energy and reducing equivalents in seeds with higher oil content. (2) Less than half of central metabolic reactions for which significant differences in flux between genotypes were identified (using 13C metabolic flux analysis) also displayed a significant shift in the concentration of either the relevant substrate or reaction product. These metabolites can be used as marker. (3) With increasing flux through glycolysis and lipid synthesis across various genotypes, levels of most glycolytic intermediates decrease significantly. This indicates that mechanisms of allosteric control are of major importance in controlling the flux partitioning between starch and lipid. Cyclic AMP and sucrose-6-phosphate were identified to potentially be involved in so far unknown mechanisms of metabolic control in plants.

poster 561

Metabolomics of early germination and growth in thermosensitive Lactuca sativa (lettuce) seeds

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Lactuca sativa (lettuce) seeds exhibit thermoinhibition, an inability to germinate above a threshold temperature. Using ASTEC Q2 Oxygen Sensing Technology, monitoring the oxygen consumption time courses of individual seeds respiration during imbibition and germination has revealed distinct differences in respiration patterns for seeds imbibed at permissive and inhibitory temperatures. Seeds imbibed at lower temperatures increase their rates of oxygen consumption over time whereas at higher temperatures seeds decrease their rate of oxygen consumption to very low levels. Although respiration is significantly decreased in thermoinhibited seeds, the respiratory pathways that maintain this background metabolic activity, as well other aspects of metabolism under these conditions are unknown. Q2 measurements followed by metabolomics profiling were used to address this question. Seeds of the cultivar Salinas and of Salinas in which abscisic acid (ABA) biosynthesis during imbibition was prevented by RNAi-silencing of NCED4 were imbibed at 25 and 35˚C with and without ABA (which inhibits germination). Data were acquired for dry seeds and for seeds imbibed for 2, 12, 24, and 36 hours. Primary metabolism was investigated using GC-TOF MS with BinBase for data processing. Lipid metabolism was investigated using LC-Q-TOF MS/MS with Mass Hunter Quantitative Analysis for data processing and LipidBlast for compound annotation. Statistical analyses were performed in Statistica and in R. Primary metabolism coverage after data cleaning resulted in 488 compounds, 189 of which were named compounds, the others being unknown. Primary metabolite coverage included amino acids, organic acids, nucleosides, short-chain fatty acids, sugars, and citric acid cycle intermediates. Results showed that increases in sugar (glucose and fructose) contents that occurred in association with germination were blocked by high temperature, but not ABA. Raffinose content declined in early germination and growth and this response was dependent on temperature apart from the action of the germination inhibitor ABA. Additionally, increases in sugar phosphates were driven by thermopermissive temperatures and not through inhibition by ABA. The biological questions addressed include a comparison of primary and lipid metabolism between germinating and non-germinating seeds and whether high temperature and ABA induces the same biological effects on metabolism. This work uses a metabolomics approach to investigate a complex biological response of seeds to their environment.

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Metabolomics provides insights into lifestyle transitions in the hemibiotrophic fungal pathogen, Colletotrichum sublineolum

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Metabolomics has emerged as a powerful and effective approach to interrogate cellular biochemistry at the global level (Tugizimana et al., 2013; 2014). In this study an LC-MS-based metabolomics approach is employed to elucidate lifestyle transitions in the fungal plant pathogen, Colletotrichum sublineolum. The latter is a hemibiotrophic fungus that causes anthracnose, one of the devastating diseases of sorghum. Hemibiotrophic phytopathogens initially establish a differentiated biotrophic interaction with the host plant and later switch to a destructive necrotrophic lifestyle. The molecular mechanisms involved in the lifestyle transitions of C. sublinoelum are still not fully understood. Such insights would assist immensely in understanding the biochemical processes involved in the interactions between hemibiotrophic phytopathogens and the host plants. Different carbon sources (glucose vs. arabinose and rhamnose to simulate in planta nutrient sources) were used and inoculated with mycelial plugs from C. sublineolum grown on solid agar media. The samples were then harversted at different time points post inoculation/p.i.

(0, 3, 6, 9 and 12 days). Both extracellular and intracellular metabolites were methanol-extracted and analysed on UHPLC-HDMS. Data analyses (of the generated multidimensional datasets) were performed using statistical tools such as PCA and OPLS-DA. Multivariate statistical batch modelling was used to extract and describe the time-related metabolic variation observed from PC-analyses. The PC analyses of Pareto-scaled data (X) of both extra- and intra-cellular samples (in all carbon sources), in relation to the time points (0-12 d, p.i.) showed evidently time-point clusters (with less within-grouPoster variation), indicating differentiated metabolic profiles over time. In order to assess optimally the time-related metabolic trajectories (defining time-related events), a PLS-based approach, namely batch statistical modelling (BM) was employed. The three-way data matrix was subjected to two subsequent levels of multivariate modelling: BEM (based on PLS regression against metabolite collection time) and BLM (PCA of PLS components). The time-course trajectory analyses/plots (from BM) described optimally differentiated phases of the dominant metabolic perturbations with time (metabolic evolution patterns). These PCA-/BM-explained variations (time-course trajectories and carbon-source related) suggest thus a metabolic reprogramming, pointing to mechanisms involved in the lifestyle changes of the fungus. Gene expression analyses of selected pathogenicity-related genes of the fungus confirmed the in-time differentiated lifestyle phases (in correlation to the PCA and BM results): biotrophic vs necrotrophic. Different (m/z, Rt) ions were statistically extracted as bio-markers of the differentiated lifestyle phases and time-related/dynamic variation. Metabolite annotation of these markers is currently being carried out and metabolic network analyses will aid in the holistic description of the fungal metabolic reprogramming (as the fungus switches from biotrophic to necrotrophic lifestyle/phases) over time and in response to nutrient sources. This study will contribute to the characterization of molecular mechanisms involved in the lifestyle transitions of the fungus

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Regulation of primary metabolism in response to flooding stress as revealed by 13C-stable isotope redistribution

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Plants are sessile, unable to relocate when exposed to diverse environmental and seasonal stimuli, hence must be able to respond rapidly to survive stress conditions. Flooding or waterlogging of the soil is a common environmental condition which greatly affects crop production and quality by blocking the entry of oxygen into the soil so that roots and other below-ground organs cannot maintain respiration. During recent decades, the number of extreme floodings has strongly increased, which is especially tragic since most arable land worldwide is located in regions that are threatened by regular flooding events. One way to meet the anticipated food needs is to develop crops that are more resilient to floods. This study presents the metabolic adaptations of wild-type roots of the crop legume soybean (Glycine max) to flooding stress combining GC-TOF-MS metabolite profiles and stable isotope labeling experiments. 13C-pyruvate labelling was performed to compare metabolism through the TCA cycle, fermentation, alanine metabolism and the γ-amino butyric acid (GABA)-shunt, whilst 13C-glutamate was performed to address the metabolism via glutamate to succinate. Following these labelling experiments, the time course for the redistribution of the 13C-label throughout the primary metabolic network was analysed with GC-TOF-MS to infer the relative metabolic activities of the various constituent pathways of central carbon metabolism. Metabolite profiling confirmed that in soybean roots a short-term hypoxic treatment (up to 6 hours) already induces fermentation with an increase in lactate and alanine and several responses in most central metabolites, such as carbohydrates, glycolytic intermediates and amino acids. Serine, derived from the glycolytic intermediate 3-phosphoglycerate (3-PGA), increased; and so did phenylalanine (derived from phosphoenolpyruvate), and valine, leucine, and alanine (derived from pyruvate). The amino acids glutamate and proline that are derived from the TCA-cycle intermediate 2OG, increase during hypoxia. On the other hand, the amino acids aspartate and asparagine, derived from the TCA-cycle intermediate OAA, decrease during hypoxia. We also performed stable isotope labelling experiments to

better understand the dynamics of metabolism in operation in plant cells under hypoxic conditions. Our labelling studies confirm the activity of the alanine shunt, while revealing the parallel activity of the GABA-shunt. The bifurcation of the TCA cycle results from the inhibition of the TCA-cycle enzyme succinate-dehydrogenase under hypoxia.

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NMR-based metabolic profiling of potato for the identification of potential metabolic indicators of common scab resistance in potato tubers

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Common scab is one of the most serious soil-borne diseases of potato tubers in the world. The disease symptoms, scab-like brown spots on the skin of the tuber, severely reduce the appearance quality, resulting in considerable economic loss. The utilization of resistant cultivars is currently considered the most promising method for controlling common scab during practical potato production. However, the selection of soil-borne disease-resistant cultivars is highly time-consuming and laborious due to the need for examining underground tissues to evaluate disease severity. In this study, we applied NMR-metabolomics to the leaves of potato plants grown in an open field to characterize common scab-resistant and -susceptible cultivars, as a model case toward a possibility of metabolic marker-assisted breeding. Eight potato cultivars with different levels of resistance to common scab were used. Seed tubers were planted in a scab-infested experimental field at the NARO Hokkaido Agricultural Research Center in April 2012. Polar and semipolar metabolites were extracted from plants with different buffers, respectively. 1H-NMR spectra were recorded on an AVANCE-500 spectrometer equipped with a Cryogenic Probe (Bruker BioSpin, Karlsruhe, Germany). For non-targeted, 1H NMR-based metabolic profiling was performed using the SIMCA software (Umetrics, Umeå, Sweden) applying Parato scaling. When examining the dataset from the polar extracts using PCA, the score plot of PC1 and PC2 showed the separation of resistant and susceptible cultivars. On the other hand, such a clear separation was not observed when examining the dataset from the semipolar extracts. To highlight metabolites associated with resistance, the datasets from the polar and semipolar extracts were subjected to PSL-DA. The generated models were comprised of four and five latent variables for polar and semipolar extracts, respectively, and the models provided cumulative R2 of 0.928 and 0.975 with cumulative Q2 of 0.818 and 0.866, respectively. Variable importance plots indicated the most responsible metabolites. Rapid and easy quantitative analyses were subsequently examined in terms of the application to practical breeding programs in experimental fields. Details for candidate metabolic indicators will be presented. Resistance to potato common scab can be estimated based on the metabolic profiles.

poster 566

Non-targeted metabolomics of potato reveals potential to breed for improved content of nutrients and bioactive compounds

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Potato is the fourth most grown crop and an important global staple food. Potato contains a diverse set of nutrients and other small molecules with demonstrated effects on preventing chronic diseases in humans,

so-called “bioactive compounds.” Characterizing the presence and variation of nutrients and bioactives in potato can inform on the potential to breed for a healthier food. To characterize the type and quantity of health-related compounds in potato, raw and cooked tubers from 60 selections/cultivars were evaluated for biochemical diversity using a non-targeted metabolomics workflow. The 60 selections/cultivars spanned 4 market classes (Russet, Red, Yellow, and Specialty) and the data was interrogated to quantify variation in select nutrients and bioactive compounds within and among market class. Cooked and raw potatoes were lyophilized and metabolites were extracted using 80% methanol. UPLC- and GC-MS workflows were applied to conduct non-targeted metabolomics on the metabolite extracts. For UPLC-MS, 1µL of potato extract was injected into a Waters Acquity UPLC (water-acetonitrile gradient) coupled to a Waters Xevo G2 ESI-TOF MS acquiring in positive ion MSE mode. For GC-MS, 250µL of extract was derivatized (methoximation/ trimethylsilylation), and 1µL was injected into a Trace GC Ultra (TG-5MS column) coupled to a Thermo ISQ mass spectrometer. Peak detection, normalization, and retention time alignment was performed using XCMS and mass spectra were reconstructed using the RAMClustR algorithm. Metabolites were annotated via spectral matching to an in-house spectral database (~1800 compounds) and public metabolite databases. Non-targeted metabolomics revealed that potato biochemical diversity was influenced by selections/cultivars and market class. Additionally, potato processing (cooked vs. raw) also influenced the potato metabolome. Furthermore, our analysis revealed specific nutrients and bioactive compounds as candidates for future breeding efforts, both within and among market classes that can result in healthier potatoes. Current efforts include determination of whether nutrients and health-related compounds in raw tubers correlate with cooked tubers, negating the step to cook potatoes for future experiments. UPLC-MS and GC-MS analysis identified select nutrients and bioactive compounds as new breeding targets to develop healthier potato.

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Comparison of curcuminoid biosynthesis of Curcuma longa and its cultivars using a pathway based RNA-Seq analysis method.

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Turmeric (Curcuma longa) is a rhizomatous plant of the ginger family, Zingiberaceae. Curcuminoids are secondary metabolites and primary active constituents of turmeric. They are accumulated in the rhizomes of Curcuma species which have long been used as spice and herbal medicine in many Asian countries and also has been identified to be of great medicinal values (anticancer, antiphlogisitc and anti oxygenic properties). Currently, because of increasing demand in the pharmaceutical and food industries, there is a pressing need to understand the dynamics of the productivity of curcumin and curcuminoid in the genus Curcuma. In this study we tried to compare the gene expression differences from two wild strains and two cultivars to understand the synthesis mechanism. We analyzed metabolism of curcuminoid in the wild type of C. longa and its two cultivars; C. longa Ougon and C. longa Sekiyou, moreover, a wild turmeric C. aromatica is also analyzed in order to evaluate their difference in their curcuminoid biosynthesis. Using the RNA-Seq method, we analyzed gene expression levels of samples taken from both leaves and rhizomes of all four specimens. We also compared gene sets along with the metabolic pathway. Namely, we selected every triplet of enzymes adjacent in the metabolic pathway from glycolysis to curcuminoids, and evaluated their expression differences between specimens in order to find significant changes to understand enhanced curcuminoid synthesis in the cultivars. Since the amount of Curcuminoids in rhizomes were much higher in the two cultivars (Ougon and Sekiyou) compared with the two wild types (C. longa and C. aromatic), first we compared gene expression levels of the two cultivars to the two wild types. We found only a few genes significantly changed their expression. Next we analyzed gene expression difference between leaves and rhizomes in order to understand how curcuminoids were synthesized and accumulated to their rhizome. We identified 284 genes that were significantly uPoster

regulated and 421 genes that were down-regulated in the leaves of all specimens. In the uPoster regulated genes, the enzymes in the central metabolic pathways such as glycolysis and the TCA cycle were enriched. Finally, we compared expression difference along with the pathways of primary metabolisms between the cultivars and wild types, in leaves and in rhizomes respectively. Because the enzymes involved in the same metabolic pathways often regulated by the same transcriptional factors, we can expect that the expression of such genes correlated each other. The result of pathway based expression analysis showed that the enzymes in the both shikimate pathway and the phenylalanine biosynthesis were significantly uPoster regulated in the leaf of C. longa Ougon and C. longa Sekiyou, compared to those of C. longa and C. aromatica. On the other hand, in the rhizome, the expression of enzymes in the sikimate pathway to the synthesis of an intermediate metabolite Chorismate, were not significantly changed, while the phenylalanine biosynthesis after Chorismate were upregulated. This suggests that the enhanced production of curcuminoid in the cultivars of C. longa depends on the biosynthesis of precursor, such as Chrorismate, in leaves, and the downstream of curcuminoid synthesis and accumulation proceed in their rhizomes. Gene set analysis that focus on adjacent enzymes along with the pathway in order to evaluate the detail metabolic behavior.

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NMR metabolomics to investigate Annona muricata root resistance to Meloidogyne javanica

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Root-knot nematodes Meloidogyne spp. are the most common nematodes responsible for economic losses in different crops and the use of synthetic nematicides as a mean of control has been causing serious damage to the environment. Therefore, the search for natural succedaneums is increasing. The resistance of some species of the Annona genus to nematodes, as well as their nematicidal activity, has been observed and reported. However, the resistance mechanism and the metabolites associated have not been identified yet. Here, we describe the use of 1H NMR spectroscopy in combination with multivariate data analysis to study metabolic changes in plants of Annona muricata exposed and not exposed to the nematode Meloidogyne javanica. Thirty individual plants of A. muricata were inoculated with M. javanica J2. Next, leaves and roots of plants 1, 2, 4, 20 and 30 days after infection were cut, freeze-dried and extracted with 50% MeOH-d4 in buffer (90 mM KH2PO4, pH 7) containing 0.05% TSP (trimethylsilylpropionic acid sodium salt, w/v) for NMR analysis. An aqueous solution was inoculated in the control group which received the same extraction treatment. The 1H NMR spectra from leaves and roots of A. muricata were reduced to ASCII files. The principal component analysis (PCA) was applied. The non-inoculated extracts were submitted to the in vitro nematicidal assay against juveniles of Meloidogyne javanica. The metabolomic study of the root extracts revealed alterations due to the natural maturation process of the plant, modeled by PC1 (30.1%), and to metabolic changes resulting from inoculation with M. javanica after 24 hours of exposure, modeled by PC3 (17.8%). This last observation suggests that a rapid defense response occurs at the infestation site, known as the hypersensitive response (HR). The results suggest that Annona muricata resistance to the Meloidogyne javanica nematode may by associated to a hypersensitivity reaction. Several resistance-related metabolites could be identified in roots with the joint analysis of 1D-and 2D NMR and the loadings of PC3, including dopamine, xanthine and acetogenins. A. muricata roots extract has shown nematicidal activity against M. javanica J2, what also suggests that a pre-formed mechanism of defense can be supporting the resistance observed. For the first time, induced and pre-formed defense mechanisms and their related metabolites were reported for the Annona muricata.

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Integration of RNAseq with 1H NMR Data sets Reveals Differential Regulation of Soybean Metabolism in Response to Rhizoctonia Foliar Blight Disease

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The developments in multi-level large-scale data acquisition and their integration within the context of systems biology enable the understanding of plant metabolism and its regulation in response to stimuli, such as fungal pathogens. Understanding plant defence to pathogens is important for applications in biotechnology, plant breeding, and crop protection. However, currently, no standard pipelines for integration between different levels of omics data exist. Here we have undergone the task of developing such approach for the integration of NMR metabolomics with RNAseq data sets. As model the plant-pathogen pathosystem soybean-Rhizoctonia solani AG-1-1A, the causal agent of foliar blight (RFB) disease was used. Two-week-old soybean leaves were detached and infected or not with R. solani. After 24 hours they were harvested and metabolites and mRNA were extracted. Metabolic profiles of infected, not infected (controls) and pure cultures of Rhizoctonia were recorded performing 1H NMR analysis and transcripts were analyzed using the Illumina HiSeq 2500 platform. Transcripts were aligned to the soybean and Rhizoctonia genomes using Bowtie 2 and the transcriptomes using TopHat2. Transcript differential expression was analyzed using the R package DESeq. The discovery of biomarkers was based on orthogonal projections to latent structures discriminate analysis (OPLS-DA), whereas O2PLS was applied for the integration between the two data sets and the discovery of the joined systematic variation. Application of OPLS-DA showed a strong discrimination between control and infected leaves, which is indicative of the substantial changes in their metabolomes following infection. Additionally, application of O2PLS with the applied transformations for 1H NMR and RNAseq data sets, enabled their integration, providing information on the metabolism regulation of soybean when it is under attack by Rhizoctonia. Transcriptomics revealed increases in transcripts encoding genes of soybean's phenylpropanoid, terpenoid and flavonoid pathways, and decreases in transcripts encoding genes of photosynthetic pathway. In total, 258 genes were differentially expressed in response to RFB disease, of which 203 were uPoster regulated and 55 were down-regulated. Fluctuations in starch and sucrose biosynthetic pathways, as well as the TCA and glycolysis pathways were also observed. On the other hand, NMR analysis revealed changes in the levels of metabolites involved in amino acid, TCA, glycolysis as well as starch and sucrose metabolic pathways. O2PLS analysis cross-validated NMR and RNAseq data by revealing shifts in the primary metabolic pathways towards precursors for the secondary metabolic pathways and decreases in photosynthetic products. Overall decreases in metabolites belonging to carbohydrates and amino acids were observed plausibly suggesting their utilization for the biosynthesis of secondary metabolites such as, glyceollins, cinnamaldehyde, coumarinate and vitamin E, as implied by their increased transcript abundances. Integration of metabolomics and trasncriptomics data sets to explore potentially relevant biological plant-microbe interactions and candidate biomarkers associated with disease.

poster 571

Metabolomics in Pesticide R&D: Probing Fungal Resistance Mechanisms to Benzimidazole Fungicides Performing 1H NMR Metabolomics

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The agrochemical industry represents the backbone of the agricultural sector world-wide. The improvement of food production, increasing concerns over food and environmental safety, and the

emergence of resistant to pesticides pests and pathogens, are among the major challenges that the sector is facing. The latter results in heavy yield losses due to the decreased efficacy of the applied crop protection agents. Within this context, the understanding of changes at the metabolome level and their correlation to the observed resistance is important for combating this issue, and represents a newly emerged and promising field in pesticide R&D. Here, we have developed a robust 1H NMR metabolomics-based protocol for the discovery and study of Fusarium graminearum resistance mechanism(s) to benzimidazole fungicides. The sensitive strain of Fusarium graminearum CBS 110261 (WT) and four carbendazim-resistant isolates; two bearing point mutations in target β2-tubulin gene (FG-3 and FG-6) and two of unknown resistance (FG-1 and FG-2), which were obtained from the WT by mutagenesis, were used. The stains were grown for 10 days on cellophane on PDA amended or not with 2 μg mL-1 carbendazim (MBC) (sub-lethal concentration for WT) at 25oC. Mycelia were harvested, lyophilized, and extracted with D2O. Extracts were analyzed using a Bruker 500MHz analyzer and data were processed in order to be subjected to multivariate analysis. Orthogonal projections to latent structures-discriminate analysis (OPLS-DA) was applied for the detection of trends and biomarkers and the correlation between metabolome and genome. Results of OPLS-DA revealed that mutation(s) resulted in distinct metabolic profiles of the strains being analyzed, which is indicative of the impact that they have on the fungal metabolism and its regulation. In controls, the metabolic profiles of the strain FG-6 were lying closer to the WT compared to the rest strains indicating that the mutation(s) of this strain had a small impact on its metabolism. Interestingly, the strains FG-1 and FG-3 shared similar NMR profiles, which plausibly indicates the presence of analogous mutation(s) leading to the observed changes in the metabolome level. Treatment with 2 μg mL-1 MBC slightly altered the clustering observed in the controls, causing a shift of FG-1 close to FG-6. This observation in combination with the results of the analysis of controls clearly indicates that the mutation(s) for FG-1 and FG-2 have occurred in different biochemical site(s). Amino acids, carboxylic acids, and carbohydrates involved in various biosynthetic pathways were identified among the biomarkers that drive the observed discriminations. 2D NMR experiments are underway for further validation of the observed biomarkers and the biosynthetic pathways involved. The present work represents a proof of concept of the applicability of NMR metabolomics for the robust and high-throughput screening of mutations in fungi leading to resistance to fungicides, and the study of their biochemical basis. Results can be exploited in genetic engineering and crop protection for combating resistance against fungicides. Original metabolomics approach for the study of fungal resistance to fungicides based on 1H NMR spectroscopy.

poster 572

Improving and Understanding Phytoremediation Capacity of Willow (Salix purpurea L.): A Metabolomics-based Approach

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Soil decontamination is a challenging, time-consuming, and costly task. The currently applied methods such as, encapsulation, immobilization, washing, and extraction, do not meet the criteria for fast, cost-effective in situ soil remediation. At the same time soil remediation should not disturb soil microbial biodiversity and its physicochemical features. Phytoremediation has emerged as an alternative of high potential for soil remediation. Willows (Salix spp., Salicaceae) include species with high phytoremediation capacity, which can be substantially increased by their symbiosis with arbuscular mycorrhizal fungi (AMF). Within this context, we have undergone the tasks of developing a biomarker-driven selection of willow cultivars based on 1H NMR metabolomics and dissecting the effect of soil contaminants and AMF on willow's metabolism. For the development of the biomarker-driven selection, 1H NMR metabolomics was performed on leaves of ten willow cultivars planted in contaminated and non-contaminated sites. The profiles of leaves were recorded, and multivariate analyses (orthogonal projection to latent structures-discriminant analysis, OPLS-DA, and hierarchical clustering, HCA) were performed for the discovery of trends and biomarkers. Model validation was performed based on physiological parameters. For the monitoring of the global metabolism regulation of willow in response to soil contamination and AMF, an advanced, robust metabolomics/bioinformatics protocol was established by integrating 1H NMR, GC/MS, and LC/MS using an LTQ Orbitrap. An essential element of the study is the metabolite species-specific

library for willow (http://willowmetabolib.research.mcgill.ca/index.html), which accelerated metabolite identification and biological interpretation of results. The developed 1H NMR model for the biomarker-assisted selection of willow cultivars based on their phytoremediation capacity, showed an excellent discrimination between the cultivars and tight clustering based on their genotypes. The genotypic composition was the predominant discriminatory factor followed by soil contamination. Major metabolites belonging to phenolics seem to drive the observed clustering and are plausibly correlated to willow's phytoremediation capacity. This study also revealed the potential and robustness of NMR for plant metabolomics studies in the field. On the other hand, the developed metabolomics/bioinformatics protocol enabled the in-depth investigation of the effects of AMF and soil contamination on willow's global metabolism. Results unraveled the complexity of AMF-willow interaction, clearly demonstrating direct and indirect beneficial effects on host priming against external stresses as well as enhancing willow's growth and productivity, both parameters valuable in integrated phytoremediation strategies. Among others, uPoster regulation of biosynthetic pathways involved in willow responses to biotic and abiotic stresses such as, flavonoid, isoflavonoid, phenylpropanoid, and the chlorophyll and porphyrin pathways was discovered as the major responses of the plant to AMF and soil contamination. The above results could be exploited in the biomarker-assisted selection of willow cultivars and in genetic engineering programs for the improvement of willow's phytoremediation capacity. Cutting-edge metabolomics/bioinformatics protocols for willows' biomarker-assisted selection and investigation of the effects of soil contamination and AMF on their metabolism.