13th Annual Conference of the Metabolomics Society

METABOLOMICS 2017

BRISBANE, AUSTRALIA June 25-29

CONFERENCE ABSTRACTS

METABOLOMICS SOCIETY

Agenda at a Glance

MONDAY

	M1	M2	M3	M4
8:00–19:00	REGISTRATION OPEN			
10:30–12:00	**Workshop 6:** Hybrid Ion Mobility MS	**Workshop 7:** Role of Metabolomics for Health & Diet Research	**Workshop 8:** Data Sharing, Standardisation and Workflow for reproducible analysis in Metabolomics	**Workshop 9:** EMN Workshop — Statistical Considerations and Pathway Analysis Strategies
10:15–10:30				
10:30–12:00	**Workshop 10:** Advances in High Throughput Targeted Metabolomics Analysis	**Workshop 11:** EMN Workshop — Career Development	**Workshop 12:** Metabolite Identification Annotation	**Workshop 13:** Clinical Biomarker Detection
13:15–15:00	**Welcome and Opening Plenary Session:** Krishna Mahadevan – Great Hall 2			
15:00–15:30	TEA AND COFFEE BREAK — FOYER			
15:30–17:10	Systems Biology Methods to Characterise Biological Systems		Model Organisms	Diabetes and Cardiovascular Disease
17:15–18:45	WELCOME RECEPTION AND POSTER SESSION 1 — EXHIBIT HALL			
19:00–19:45				**Asia-Oceania Delegates Reception**

TUESDAY

	M1	M3	M4
8:00–19:00	REGISTRATION OPEN		
07:45–08:30	**Plenary Session 2:** Hanne Bertram — Great Hall 2		
9:45–10:30	BREAK — EXHIBIT HALL		
10:30–12:00	The Staple Foods	Advances in Spatial Metabolomics	Pregnancy, Infants and Children
12:10–13:30	LUNCH — EXHIBIT HALL / PLATINUM SPONSOR PRESENTATIONS		
12:20–13:20	**Sponsor Presentation:** Agilent Technologies	**Sponsor Presentation:** Shimadzu Australasia	**Sponsor Presentation:** Waters Corporation
13:30–15:00	Marine and Microbial Natural Products	Advances in Statistics & Machine Learning	Infectious Disease
15:00–15:30	BREAK — EXHIBIT HALL		
15:30–17:00	Genome-scale Modelling & Flux Balance Analysis	Frontiers in Lipidomics	Nature's Apothecary
17:15–18:45	**Poster Session 2** — Exhibit Hall		
19:00–20:30			**EMN Reception** — Advance Sign-Up Required

WEDNESDAY

	M1	M3	M4
8:15–19:00	REGISTRATION OPEN		
8:45–9:45	**Plenary Session 3:** Anthony Carroll — Great Hall 2		
9:45–10:30	BREAK — EXHIBIT HALL		
10:30–12:10	Natural Products and Metabolomics — Advancing Two Fields	Multi-omics / Systems Biology	Cancer
12:10–13:30	LUNCH — EXHIBIT HALL / PLATINUM SPONSOR PRESENTATIONS		
12:20–13:20	**Sponsor Presentation:** Thermo Fisher Scientific	**Sponsor Presentation:** Bruker Corporation	**Sponsor Presentation:** SCIEX
13:30–15:00	New Tools in Natural Product Annotation	Environment: Dipping into the Water	Diet, Weight and Physical Activity
15:00–15:30	BREAK — EXHIBIT HALL		
15:30–17:00	Metabolite Identification, Libraries & Cheminformatics	Wine, the Great Gift from Bacchus	Population-based Metabolomics Research
17:15–18:45	**Poster Session 3** — Exhibit Hall		
19:30–22:30	CONFERENCE DINNER — PLAZA BALLROOM		

THURSDAY

	M1	M3	M4
8:15–15:30	REGISTRATION OPEN		
8:45–9:45	**Plenary Session 4:** Roy Goodacre — Great Hall 2		
9:45–10:45	**Poster Session 4** — Exhibit Hall		
10:45–12:25	Data Mining & Computational Workflows	Edibilomics	Metabolomics in Health & Disease I
12:25–13:45	LUNCH — EXHIBIT HALL / PLATINUM SPONSOR PRESENTATIONS		
12:35–13:35		**Sponsor Presentation:** Metabolon	
13:45–15:15	Quantitative Metabolomics and Data Quality	Plants	Metabolomics in Health & Disease II
15:15–15:30	Gather in Great Hall 2 for Closing Plenary Session		
15:30–16:30	**Plenary Session 5**: Debra Meyer — Great Hall 2		
16:30–17:00	**Closing Plenary Session**		

Building Bridges: Systems Biology / Metabolic Modelling

Building Bridges: Natural Products / Metabolite Identification

Advancing the Field: Methods, Data Analysis and Visualisation

Environmental Metabolomics, Plant and Model Organisms

Metabolomics in Health and Medicine

Metabolomics in Food Research — Edibilomics

TABLE OF CONTENTS

CONFERENCE THEMES

SPONSOR ADVERTISEMENTS

O-66	A Graphical Cellular Dashboard for Analysis of Metabolomics Data

PRESENTING AUTHOR: *Suzanne Paley, SRI International, United States*

CO-AUTHORS: *Peter Karp*

We present a visual cellular dashboard for graphical interactive analysis of metabolomics datasets. The tool organizes metabolomics data biologically, into individual metabolic pathways and groupings of related pathways. The dashboard enables the user to easily assess the activation levels of different areas of metabolism, from gross areas such as carbohydrate degradation, to specific pathways such as proline biosynthesis. The hierarchical organization of the dashboard is its key organizing principle. At the highest level, the dashboard is organized into four panels: Biosynthesis, Degradation, Energy Metabolism, and Other. Each panel consists of a series of graphs, such as for amino acid biosynthesis and cofactor biosynthesis. Each graph depicts all measurements for metabolites within those metabolic pathways. The user can click on any graph to generate a new window containing an expanded panel that includes a graph for each component of the previous graph, e.g., clicking on the graph for amino acid biosynthesis produces individual graphs showing metabolite levels within every amino acid biosynthetic pathway. Clicking on one of those amino acid graphs drills down further to produce a graph showing the levels of each individual metabolite within the pathway. From here, users can also view the pathway diagram overlaid with metabolite data. This tool is available at the BioCyc website, http://biocyc.org.

O-72	Assessing Proficiency in Mass Spectrometric Lipid Measurement and Annotation using a NIST International Lipidomics Interlaboratory Comparison Exercise

PRESENTING AUTHOR: *Candice Ulmer, National Institute of Standards and Technology (NIST), United States*

CO-AUTHORS: *Christina Jones, Alan Heckert, Jeremy Koelmel, John Bowden*

Advances in mass spectrometric techniques have increased the number of lipid species detected. Measuring lipid profile shifts has promising potential for personalized medicine and biomarker discovery applications. However, there is limited harmonization in the lipidomics community regarding sample handling/preparation, data processing, and lipid identification strategies. Our lipidomics interlaboratory study was designed to (1) highlight challenges and sources of variance in the lipidomics workflow, (2) provide lipid concentrations and associated uncertainty ranges derived from various matrices, diverse methodologies, and institutions to serve as benchmark values for quality assurance in lipid measurement, and (3) develop software to standardize lipid measurement. Domestic (n = 18) and international laboratories (n = 14), representing academia, industry and government, quantitated 1527 unique lipid species levels in triplicate (nmol/mL) for 52 lipid classes from the following samples: SRM 1950 (Metabolites in Frozen Human Plasma), SRM 2378 series 1-3 (Fatty Acids in Frozen Human Serum), and an Avanti Bovine Liver Extract. Consensus means and corresponding uncertainty values were calculated for 330 lipids (annotated at lipid species level) in SRM 1950 and compared to published data by LIPID MAPS. Z-scores were used to assess proficiency in quantitation by laboratory and by the individual lipid species. The following laboratory-provided meta-data was scrutinized alongside lipid measurement for each material: laboratory profile (targeted or global), sample extraction, sample introduction/separation, mass spectrometry instrument, and data processing protocols. Two resulting software, LipidQC and LipidPioneer, were also designed from observations in the interlaboratory study to address quality assurance and accuracy in lipid exact-mass calculations, respectively.

O-77	MAIMS: a GAIMS alternative for the deconvolution of UDP-N-acetyl-D-glucosamine 13C mass isotopologue profiles

PRESENTING AUTHOR: *Dries Verdegem, Metabolomics Expertise Center - VIB / KU Leuven, Belgium*

CO-AUTHORS: *Hunter Moseley, Wesley Vermaelen, Abel Acosta Sanchez, Bart Ghesquière*

In 2011, the GAIMS algorithm for the deconvolution of the UDP-GlcNAc 13C mass isotopologue profile into the contribution of specifically labeled glucose, ribose, acetyl and uracil moieties upon U-13C-glucose administration was presented. O-GlcNAc signaling has been recognized as an important factor in the maintenance of metabolic homeostasis through its cell signaling, gene transcription and translation actions and epigenetic reprogramming. In line with this, the precursor for O-GlcNAcylation (UDP-GlcNAc), the production of which relies on the activity of several metabolic pathways, has been put forward as a key nutrient sensing metabolite. Consequently, the outlook of unraveling the UDP-GlcNAc isotopologue profile into relative fluxes through several essential biochemical pathways (e.g. glycolysis, pentose phosphate pathway, TCA cycle and pyrimidine biosynthesis) using GAIMS was promising and its development a significant contribution to the field of Metabolic Tracer Analysis (MTA), which deals with the qualitative interpretation of stable isotope incorporation patterns. Unfortunately, GAIMS was presented as a proof-of-concept algorithm only and despite its usefulness never made available to the MTA community as a usable tool. We have therefore implemented the freely available MAIMS, a tool inspired by the original GAIMS algorithm and providing identical functionality. To solve the described deconvolution problem, which is in essence a non-convex optimization problem, MAIMS relies on Multistart metaheuristics, in which the optimal solution is sought by performing several local searches with random initialization. Applying MAIMS on UDP-GlcNAc measurements in proliferating endothelial cells (ECs) confirmed recently discovered metabolic preferences in these cells but also resulted in novel metabolic insights.

O-88	The whole is easier than the parts: Improving molecular formula identification using Gibbs sampling on fragmentation trees

PRESENTING AUTHOR: *Marcus Ludwig, Friedrich-Schiller-University Jena, Germany*

CO-AUTHORS: *Kai Dührkop, Louis-Felix Nothias-Scaglia, Sebastian Böcker*

Liquid Chromatography Tandem Mass Spectrometry (LC-MS/MS) is one of the predominant experimental platforms for untargeted metabolomics, but searching acquired tandem spectra in spectral libraries will only identify the usual suspects. SIRIUS 3.4 combines isotope pattern and fragmentation pattern analysis, and enables de novo identification of molecular formulas. While showing excellent performance for compounds with mass below 500Da, larger compounds (potentially) containing halogens prove to be challenging due to the tremendous number of candidate molecular formulas. We introduce ZODIAC, a computational tool utilizing the inherent dependence between compounds to improve identification quality. Metabolites produced by organism(s) are derived from multiple, but limited, biogenetic pathways. As a result, metabolites in a biological sample are likely to share (structural) properties. Hence, correct molecular formula identifications are expected to be more similar (measured by fragmentation tree similarity) than incorrect ones. We derive a probabilistic model where related explanations for different compounds support each others' plausibility. Using Gibbs sampling, we estimate the best molecular formula for each compound using the posterior probability. ZODIAC also allows to incorporate spectral library hits as "anchor" within the network of explanations, further boosting identification quality. We applied ZODIAC to Euphorbia dendroides LC-MS/MS data. We find that the identification rate for 48 compounds with mass above 700Da improves from 43.8% when estimating each compound's molecular formula independently using SIRIUS 3.4, to 85.4% using ZODIAC. By adding MS/MS anchors, performance was improved to 97.9%. Hence, Zodiac enables comprehensive molecular formula identification, forming the basis for reliable downstream analysis, including structural elucidation.

O-127	Simultaneous Quantification of 124 Amino Metabolites in 22 Metabolic Pathways Using UH-PLC-MS/MS

PRESENTING AUTHOR: *Huiru Tang, Fudan University, China*

CO-AUTHORS: *Jin Wang, Yulan Wang, Lihong Zhou*

Metabolites containing amino groups cover multiple pathways and play important roles in redox homeostasis and biosyntheses of proteins, nucleotides and neurotransmitters. Here, we report a new method for simultaneous quantification of 124 such metabolites in more than 20 metabolic pathways in a single short run. This is achieved by derivatization-assisted sensitivity enhancement (DASE) with 5-aminoisoquinolyl-N-hydroxysuccinimidyl carbamate (5-AIQC) followed with comprehensive analysis using ultra-high performance liquid chromatography and electrospray ionization tandem mass spectrometry (UHPLC-MS/MS). In an one-pot manner, this quantification method enables simultaneous coverage of more than 20 important metabolic pathways including protein biosynthesis/degradation, biosyntheses of catecholamines, arginine and glutathione, metabolisms of folate-associated homocysteine, taurine-hypotaurine et al. Compared with the reported ones, this method is capable of simultaneously quantifying thiols, disulfides and other oxidation-prone analytes in a single run and suitable for quantifying aromatic amino metabolites. This method is also much more sensitive for all tested metabolites with LODs well below 50 fmol (at sub-fmol for most tested analytes) and shows good precision for retention time and quantitation with inter-day and intra-day relative standard deviations (RSDs) below 15% and good recovery from renal cancer tissue, rat urine and plasma. The method was further applied to quantify the amino metabolites in silkworm hemolymph from multiple developmental stages showing its applicability in metabolomics and perhaps some clinical chemistry studies. It is expected that this method would be applicable in foodomics as well.

O-147	Automated Real-Time Quality Control of LC-MS Metabolomics Data

PRESENTING AUTHOR: *Jan Stanstrup, Steno Diabetes Center Copenhagen, Denmark*

CO-AUTHORS: *Stinus Lindgreen*

Robust results and sound conclusions can only be achieved when high quality data are available. In metabolomics this is particularly difficult to achieve since the large datasets necessary require long acquisition times – typically weeks to months. Because of the production time, critical problems can remain hidden until initial data analysis, at which point hundreds of samples might have been analyzed. At this time it will be expensive and time-consuming, if at all possible, to re-analyze the samples. The common practice of spot-checking during the run is unreliable for identifying issues that, while non-obvious, can have detrimental effects on data consistency. As metabolomics is maturing to the point where it can form the basis of clinical diagnostic platforms, a definitive requirement will be tight control of data quality. To this end, we have developed an automated system that tracks quality parameters and generates dynamic online reports in real time. Common problems can be detected as the samples are being analyzed, thereby giving the operator the ability to identify and correct any issues immediately. The system tracks selected compounds for retention time drift and line broadening, loss of m/z calibration and sensitivity loss. It is also possible to concurrently monitor levels of known contaminants and warn the operator if contamination reaches unacceptable levels. The system is fully open source, modular, extendable and based on R allowing bioinformaticians to tune or extend the system to specific needs.

O-163	Using standardised drift-tube ion mobility to enhance non-targeted assessment of the wine metabolome

PRESENTING AUTHOR: *Tim Causon, University of Natural Resources and Life Sciences, Austria*

CO-AUTHORS: *Dragana Petrusheva, Elena Bogeva, Violeta Ivanova-Petropulos, Stephan Hann*

Liquid chromatography with drift-tube ion mobility spectrometry-mass spectrometry (LCxIM-MS) is emerging as a powerful addition to existing LC-MS workflows for addressing a diverse range of metabolomics-related questions [1,2]. Importantly, excellent precision under repeatability and reproducibility conditions of drift-tube IM separations [3] supports the development of non-targeted approaches for complex metabolome assessment such as wine characterisation [4]. In this work, fundamentals of this new analytical metabolomics approach are introduced and application to the analysis of 90 authentic red and white wine samples originating from Macedonia is presented. Following measurements, intersample alignment of metabolites using non-targeted extraction and three-dimensional alignment of molecular features (retention time, collision cross section, and high-resolution mass spectra) provides confidence for metabolite identity confirmation. Applying a fingerprinting metabolomics workflow allows statistical assessment of the influence of geographic region, variety, and age. This approach is a state-of-the-art tool to assess wine chemodiversity and is particularly beneficial for the discovery of wine biomarkers and establishing product authenticity based on development of fingerprint libraries. References 1. Stow, S.M., Causon, T.J., Zheng, X., Kurulugama, R.T., Mairinger, T., May, J.C., Rennie, E., Baker, E., Smith, R.D., McLean, J.A., Hann, S., Fjeldsted, J.C. Submitted to Analytical Chemistry 2. Metz, T.O., Baker, E.S., Schymanski, E.L., Renslow, R.S., Thomas, D.G., Causon, T.J., Webb, I.K., Hann, S., Smith, R.D., Teeguarden, J.G. 2017. Bioanalysis, 9, 81. 3. Causon, T.J., Došen, M., Reznicek, G., Hann, S. 2016. LC-GC Europe, 29, 666. 4. Ortmayr, K., Causon, T., Hann, S., Koellensperger, G. 2016. Trends Anal. Chem., 82, 358.

O-200	MALDI Imaging of lipids and pharmaceuticals in human prostate cancer explants.

PRESENTING AUTHOR: *Paul Trim, South Australian Health and Medical Research Institute (SAHMRI), Australia*

CO-AUTHORS: *Xander Spotbeen, Bala Prabhala, O. Johan Gustafsson, Margaret Centenera, Johan Swinnen, Lisa Butler, Marten Snel*

Defining lipid perturbations in prostate cancer may lead to a better understanding of disease pathology, prognosis and therapy efficacy. A study using MALDI imaging for phospholipids and drug penetration studies using a novel tissue culture explant model of prostate cancer will be presented. Our innovative tissue explant model allows for a single tissue core obtained from surgery to be cut into small cubes and cultured under different conditions; in this case, treatment with the current clinical agent Enzalutamide or vehicle (DMSO) as a negative control. Using the same patient material allows each sample to have its own matched controls. Analysis of the cultured tissue cores using MALDI imaging has allowed us to view the spatial distribution of several distinct phospholipid species within specific pathological regions of these heterogeneous tissues, enhancing the identification of tumour lipid alterations and metabolite signatures. Further to this, we have verified that the pharmaceutical compound enzalutamide, when dissolved in the tissue culture media, completely penetrates the tissue core over a relatively short period. The work presented is underpinned by extensive untargeted lipidomic analysis, in which lipid species identified in whole tissue homogenates have been mapped using MALDI Imaging. This has provided the spatial information of individual lipid species not achievable using other techniques. Demonstrated here are techniques relevant to the wider metabolomics community adding spatial information to the metabolomics workflow.

O-212	Exploring the use of ultra-high performance supercritical fluid chromatography mass spectrometry (UHPSFC-MS) for lipidomics applications

PRESENTING AUTHOR: *Joost Brandsma, University of Southampton, United Kingdom*

CO-AUTHORS: *John Langley, Julie Herniman, Timothy Jenkins, Tony Postle*

Lipids and lipid-derived metabolites play key roles in the cellular homeostasis, metabolism and signalling of all organic life. Consequently, the applications of lipid analytical platforms are varied and wide-ranging: from disease biomarker discovery and phenotyping, to nutritional biochemistry, or the molecular basis of microbial ecology and its impact on global elemental cycles. The recent resurgence of supercritical fluid chromatography (SFC) has provided the lipidomics field with a separation technology that is not only orthogonal to conventional gas and liquid-based systems, but promises tangible benefits in terms of chromatographic resolution and analysis times. The efficacy of this technology for both targeted and untargeted lipidomics studies will be demonstrated with examples from newly-developed lipidomics assays using ultra-high performance supercritical fluid chromatography mass spectrometry (UHPSFC-MS). These include quantitative methods to measure: 1) all major neutral and polar membrane lipids in mammalian and plant/algal cells; 2) lysophosphatidic acids (LPAs) and other lysophospholipids; 3) eicosanoids; 4) cardiolipins (CLs); and 5) free fatty acids (FFAs), fatty acid methyl esters (FAMEs) and triglycerides (TAGs). In each of these examples UHPSFC offers a different, and in many cases superior, chromatographic separation to established GC or LC methods. Moreover, analysis times are short with assays taking between 2 and 10 minutes, depending on the target compound(s). The new generation UHPSFC systems are particularly well-suited to lipid analysis, providing a valuable alternative for analytes that are hard to separate by other methods, and offering increased sample throughput in large-scale lipid/metabolomics studies.

O-217	Feature extraction from high-dimensional metabolomics datasets using Knowledge Discovery by Accuracy Maximization (KODAMA)

PRESENTING AUTHOR: *Stefano Cacciatore, Imperial College, United Kingdom*

CO-AUTHORS: *Leonardo Tenori, Claudio Luchinat, Phillip Bennett, David MacIntyre*

Knowledge discovery and data mining are interdisciplinary areas focusing upon development of methodologies useful for extracting features from complex data (e.g., metabolomics) that facilitates increased knowledge of the system being studied. Here we present the application of our recently improved and optimised learning algorithm, KODAMA, to high dimensional data specifically focusing on metabolomics datasets. The core idea of the algorithm is to use only the data set as input (no a priori knowledge is needed) and apply an iterative procedure that permits classification with a high cross-validated predictive accuracy. The cross-validated accuracy can be calculated by using any supervised classifier. External class information (e.g., patient metadata) can now be integrated in KODAMA before performing the iterative procedure thereby supporting an additional semi-supervised approach for highlighting otherwise hidden features of interest. KODAMA represents a valuable tool for performing feature extraction on noisy and high-dimensional datasets and can be used to identify metabolite features associated with the generated output and the related patient information that are easily interpretable for the user.

O-249	NMR Quantitates Whole Blood, Tissue and Even MS-Detected Metabolites

PRESENTING AUTHOR: *Daniel Raftery, University of Washington, United States*

CO-AUTHORS: *Nagana Gowda, Danijel Djukovic, Haiwei Gu*

Conventional human blood metabolomics employs serum or plasma and provides a wealth of metabolic information. However, important metabolites such as coenzymes that are present in red blood cells are missed. We show here that 1H NMR can simultaneously quantitate 7 coenzymes and antioxidants (NAD+, NADH, NADP+, NADPH, ATP, ADP, AMP, GSH, and GSSG) in extracts of whole human blood, in addition to the nearly 70 metabolites that we quantify in serum/plasma, with essentially no additional effort. A new sample preparation method was developed for detecting these unstable species without affecting other metabolites. We similarly developed an approach to quantitate these species in 5 tissue types. Coenzyme and antioxidant levels represent a sensitive measure of cellular function in health and numerous diseases; the NMR method presented here offers new capabilities for deriving their concentrations simultaneously in numerous sample types. Quantitative NMR data can also be applied to determine MS-detected metabolite concentrations without the use of internal or external metabolite standards. Metabolite concentrations in a serum sample were determined using NMR and a TSP reference. MS peak integrals of these metabolites were then compared to MS peak integrals in 7 new samples and used to determine the metabolite concentrations. Concentrations determined by NMR and NMR-guided MS agreed extremely well (R2>0.99). However, six metabolites correlated poorly due to stability issues in their MS analysis. Expansion of the number of quantified metabolites without internal standards is continuing.

O-256	When the microbiome meets the metabolome: A Framework for integrative longitudinal analysis

PRESENTING AUTHOR: *takoua jendoubi, School of public health, Imperial College London, United Kingdom*

CO-AUTHORS: *Panagiotis Vorkas, Perrine Masson, Arnaud Wolfer, Erwan Werner, Jeremy Nicholson, Bernard Walther, Elaine Holmes, Timothy Ebbels, Robert Glen*

Metabonomics time-course experiments provide the opportunity to observe the evolution of metabolic profiles in response to internal and external stimuli. Along with other omic longitudinal profiling technologies, these techniques have great potential to complement the analysis of complex relations between variations across diverse omic variables and provide unique insights into the underlying biology of the system. However, many statistical methods currently used to analyse short time-series omic data are i) prone to overfitting or ii) do not take into account the experimental design or iii) do not make full use of the multivariate information intrinsic to the data. The model we propose is an attempt to i) overcome overfitting by using a weakly informative Bayesian model, ii) capture experimental design conditions through a mixed-effects model and iii) model interdependencies between variables by augmenting the mixed-effects model with an ARIMAX component . We present our methodology in the context of a randomized controlled trial in a rat model. In this study, comprehensive metabolic phenotyping of metformin effect was performed, by monitoring the longitudinal metabolic variations in the plasma of healthy Wistar rats. The analysis was complemented with 16S rRNA gene sequencing in order to observe concurrent changes in the gut microbiome and attempt to identify potential blood biomarkers of putative changes in the gut microbiota. Results show that mild alterations observed within the gut microbiome are associated with a cascade of changes involving the host organism metabolome and gut microbiome, possibly supporting the hypothesis of an inter-level feedback loop.

O-264	Identifying epimetabolites by mass spectrometry-based cheminformatics

PRESENTING AUTHOR: *Hiroshi Tsugawa, RIKEN, Japan*

CO-AUTHORS: *Zijuan Lai, Gert Wohlgemuth, Masanori Arita, Oliver Fiehn*

Epimetabolites are metabolites removed from canonical biochemistry pathways that can be associated with biological functions. Most often, these compounds have unknown chemical structures and are discovered in untargeted metabolomics by statistical associations. We here present a systematic workflow supporting three tasks for the identification of epimetabolites. (i) BinBase investigator: query compounds in BinBase metabolomics database for annotation records with biological metadata across 1,900 studies in over 90,000 samples. (ii) MS-DIAL: universal chromatographic feature deconvolution with high resolution mass spectrometry analytics. (iii) MS-FINDER: automatic structure elucidation for GC-EI-MS and LC-ESI-MS/MS with searching against an enzyme promiscuity library, MINE database. Importantly, our new versions are now also capable of utilizing GC-MS data although both MS-DIAL and MS-FINDER were only suitable for LC-MS/MS data. The main part of this talk is to introduce our strategy for the identification of novel epimetabolites that were discovered in very different biological studies. Second, the MS-DIAL update is featured by comparing it against other open access or commercial chromatogram deconvolution methods using identical GC-MS raw data as input. Finally, the theory and update of MS-FINDER is introduced with the comparison against alternative software including open access or commercial programs.

O-268	Curated open-access LC-Orbitrap-MS/MS spectral library of endogenous metabolites and lipids

PRESENTING AUTHOR: *Prasad Phapale, EMBL, Germany*

CO-AUTHORS: *Andrew Palmer, Dominik Fay, Ivan Protsyuk, Theodore Alexandrov*

Metabolite and lipid identification is crucial to derive biological conclusions is still a major challenge for any untargeted metabolomic studies. Even High-resolution MS measurements (accuracy < 1 ppm) are not sufficient for unambiguous identification in many cases. The gold standard for metabolite identification in metabolomics MS community is by matching fragmentation spectra (MS/MS) from authentic standards. The comprehensive LC-MS/MS spectral libraries are still not freely available due to several challenges. Here we share our 'LC-Orbitrap-MS/MS EMBL-MCF spectral library' from over 700 authentic metabolite standards which cover major endogenous biochemical pathways. We have performed LC-MS/MS analysis of over 700 authentic standards purchased from IROA, Sigma, Avanti and other sources. Class-specific protocols were used to analyze these compounds in both positive and negative ESI ionization modes. We have developed the web-browser based software Curatr, which hosts the library, facilitates the curation, and enables search and sharing (http://curatr.mcf.embl.de/). We have curated all relevant MS/MS spectra across chromatographic profiles from accurate (< 10 ppm) precursors and possible adducts. Alongside fragmentation data, we record the LC-MS protocol and collision energies used. The curated spectra and related LC-MS metadata is freely available for download in several formats. This is the first ever freely available LC-Orbitrap-MS/MS spectral library containing not only MS/MS spectra for over 700 compounds but also all metadata along with LC-MS protocol. The library is freely available online at our core facility website (www.embl.de/mcf) enabled by the Curatr web-based software. We are working on integrating the EMBL-MCF spectral library into GNPS, MassBank and MetaboLights.

O-273	Enhanced Negative Mode Ion-less Matrices for Imaging Mass Spectrometry – Assessing MALDI Matrices for Metabolite Annotation and Spatial Metabolomics in Plant and Mammalian Systems

PRESENTING AUTHOR: *Berin Boughton, University of Melbourne, Australia*

CO-AUTHORS: *Jeffrey Spraggins, Wesley Bryson, Richard Caprioli, Andrew Palmer, Theodore Alexandrov, Antony Bacic, Ute Roessner*

Imaging Mass Spectrometry (IMS) is rapidly maturing as an advanced method for profiling the spatial distribution of metabolites in tissues. A new online data analysis platform, METASPACE, allows automated annotation of metabolites unlocking the potential of IMS. Matrix Assisted Laser Desorption Ionization (MALDI) is the most common IMS platform and uses chemical matrices to aid ionization. Current matrices used for negative ionization are limited or possess drawbacks. A suitable matrix for negative ionization is a non-reactive organic base, with a high pKa and high UV absorbance that does not generate low molecular weight matrix ions or adducts complicating interpretation. Proton Sponge, 1,8-Bis(dimethethylamino)naphthalene (DMAN) was reported by Shroff et al. (2009) as an Atmospheric Pressure-MALDI matrix possessing these qualities but is unsuitable for routine MALDI-MS due to sublimation under high vacuum environments of most MALDI instruments. Here, we report the analogue, 1,8-Bis(1-pyrrolidinyl)naphthalene (BPYN) as an enhanced negative mode ion-less matrix for high mass and high spatial resolution Fourier Transform Ion Cyclotron Resonance (FTICR) MALDI-IMS of plant and mammalian tissues; and demonstrate the capacity to conduct positive mode ionization using the same matrix. To validate performance, we compared BPYN to common negative and positive ionization mode matrices on serial sections of rat brain. For each matrix, metabolite annotation was performed using METASPACE at False Discovery Rate of 0.2 and Metabolite Signal Match score cut-off of 0.1 for each matrix. We found equivalent or better performance for the analysis of fatty-acids, glycerophospholipids and gangliosides when using BPYN compared to common matrices in use.

O-301	**Automated metabolite substructure recommendation from unexplained spectra using pattern mining**

PRESENTING AUTHOR: *Aida Mrzic, University of Antwerp, Belgium*

CO-AUTHORS: *Pieter Meysman, Wout Bittremieux, Kris Laukens*

The identification of metabolites from mass spectral data remains the largest bottleneck in advancing metabolomics. Despite the existence of several reliable tools for structural elucidation of known metabolites, there is still no reliable bioinformatics tool that can identify previously unseen compounds. Here, we present a tool based on an automated method for substructure recommendation from mass spectra using pattern mining. Our approach identifies parts of unknown metabolites based on previously seen recurring substructures. This approach does not require any prior information regarding the metabolites to be identified, and therefore can be used for the partial identification of unknown unknowns. We generated substructure recommendations for the MS/MS spectra from 58 metabolites from the previous two CASMI challenges. For all but one CASMI compound, the recommendations were significantly enriched with correct substructures. As this approach is best used complementary to existing metabolite identification tools, it was combined with the MAGMa search engine, which uses a structural database and was the winner of CASMI2014. Combining the substructure recommendations with the top 50 MAGMa identifications, we improved the ranking of the correct structure for 55% of the compounds (in addition to 20% top ranked compounds which retained their rank), resulting in a significant increase in correct metabolite identifications. The substructure recommendation procedure is implemented in an online tool, where users can upload their own MS/MS spectra to get recommendations.

O-309	**Multivariate modelling with unbiased variable selection**

PRESENTING AUTHOR: *Carl Brunius, Chalmers University of Technology, Sweden*

CO-AUTHORS: *Lin Shi, Rikard Landberg*

Metabolomics and other 'omics' technologies in general result in large, high-dimensional experimental data, which can be used e.g. for mechanistic investigations into pathophysiological processes or to discover biomarkers. To cope with the data structure, multivariate (MV) modelling is frequently used. However, the number of variables will normally far outweigh the number of observations and modelling thus requires reliable validation. Partial least squares analysis (PLS) and random forest (RF) are able to handle large, high-dimensional data with collinear variables and are widely applied for scientific MV computations. Although designed for MV analyses, these methods benefit from a compact data structure with non-redundant predictors in terms of decreased computation times, improved predictive performance, reduced likelihood of overfitting and simplified data interpretation. Variable selection is thus an important step to obtain parsimonious MV models. We have developed the MUVR algorithm, an approach for unbiased variable selection in which a nested cross-validation procedure, i.e. with an inner and outer cross-validation loop to separate data into training, validation and testing partitions to minimise overfitting, was extended to also tune the number of variables. Integrating variable selection with crossvalidation provides simultaneous solutions to the minimal-optimal and all-relevant problems, i.e. finding on one hand the minimal solution with non-redundant data and on the other all relevant features. The MUVR algorithm allows for both PLS and RF core modelling and supports regression, classification and multilevel analyses. The algorithm was compared, using authentic data, to several other variable selection techniques, both side-by-side and in combinations.

O-346	Quantitative Lipidomics and Discovery Metabolomics Applied to Human Sebum

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CO-AUTHORS: *Richard Robinson, Kelli Goodman, Anne Evans, Luke Miller*

Sebum is a lipid-rich secretion that coats the surface of human skin and contributes to antimicrobial defense, water retention, photoprotection, and wound healing. The human sebum lipidome is uniquely characterized by the presence of wax esters and squalene, as well as free fatty acids and di- and triacylglycerols. Past lipidomic studies of sebum have been limited to bulk fatty-acid measurements or, more recently, to complex lipids identified only at the sum composition level and without absolute quantification across lipid classes. Here, we developed the first analytical method that simultaneously quantifies and resolves the molecular composition of the major lipid classes of human sebum. We collected sebum samples from 60 healthy volunteers and subjected them to organic solvent extraction followed by automated flow injection into a QTrap 5500 mass spectrometer operated in Multiple Reaction Monitoring mode. Starting from a broad combinatorial list of >2,500 molecular species, we identified approximately 1,000 specific lipid species that reproducibly account for the vast majority of total signal in each lipid class, and included at least one internal standard per class. We went on to validate the analytical reproducibility, recovery, and linearity of the assay. In addition, we performed the first global metabolomic analysis of sebum on Metabolon's established Discovery HD4 platform, revealing significant levels of ~50 metabolites including amino acids, nucleotide metabolites, microbial metabolites, and xenobiotics. Together, these optimized, high-throughput methods provide unprecedented insight into the lipidomic and metabolomic composition of human sebum.

O-352	MicrobiomeAnalyst - a web-based tool for comprehensive statistical, visual and functional analysis of microbiome data

PRESENTING AUTHOR: *JIANGUO XIA, McGill University, Canada*

CO-AUTHORS: *Achal Dhariwal, Jasmine Chong, Salam Habib, Luis Agellon*

MicrobiomeAnalyst (http://www.microbiomeanalyst.ca) is a novel web-based tool that integrates the latest progress in statistics and visualization techniques, coupled with high-quality knowledge bases to enable comprehensive analysis of various data sets generated in microbiome studies. MicrobiomeAnalyst contains four modules - the Marker Data Profiling (MDP) module offers comprehensive support for community diversity profiling, differential abundance analysis and prediction of metabolic potentials for 16S rRNA marker gene data; the Shotgun Data Profiling (SDP) module supports diverse functional profiling, exploratory data analysis, as well as powerful metabolic network visual analytics for metagenomics or metatranscriptomics data; the Taxon Set Enrichment Analysis (TSEA) module allows researchers to easily interpret taxonomic signatures through enrichment analysis against >300 taxon sets manually curated from the literature and public databases; the Projection with Public Data (PPD) module allows users to visually explore their data with a public reference data for pattern discovery and biological insights. MicrobiomeAnalyst has been developed based on our high-performance MetaboAnalyst (http://www.metaboanalyst.ca) framework to support real-time interactive data analysis for ~100s of users. Finally, MicrobiomeAnalyst complements MetaboAnalyst with novel features for multi-omics data integration through biological networks and several advanced multivariate statistics.

O-414	Metabolomics on the cloud using workflows: the PhenoMeNal approach

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A bioinformatics analysis of metabolomics data can be demanding in a number of ways: due to the variety of metabolomics software tools, complexity of installing them on a High Performance Computing (HPC) environment, expertise needed to operate such software, scalability issues and the intricacies of moving large data produced in the context of metabolomics from ingestion point to the HPC site, among others. Here we present the PhenoMeNal computational infrastructure for metabolomics, which is built to tackle these problems. We rely on proven workflows environments, such as Galaxy, to facilitate the use of complex metabolomics software. To avoid installation issues, the tools and environments that we provide are packaged in software containers (Docker), and can be used as well independently of our complete installation. The problem of running tools in different development and production environments is tackled by managing our containers through a Kubernetes container orchestrator layer, which means that the higher tier of PhenoMeNal can also be deployed on any Kubernetes installation. Finally, we provide an automated manner of deploying all the infrastructure through a modern web interface on different cloud providers (i.e. Google, Amazon and OpenStack installations), where the user only provides his/her credentials to have a small private running cluster with all the tools available in a matter of minutes. Currently we deliver more than 30 metabolomics tools through our customized Galaxy workflow environment which we hope will be useful for the community, and we illustrate how to make more tools available on it.

O-425	The use of ion mobility coupled with high resolution mass spectrometry to improve separation and identification of lipid biomarkers in metabolic diseases

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A major challenge of LC-MS of complex lipid mixtures is the separation/identification of isobaric compounds, which often have similar chromatographic properties and fragmentation data can be inconclusive. Ion mobility (IM) separates isobaric ions based on their mobilities through an inert gas, with mobility correlated to collisional cross sections (CCSs) of molecules, which in turn relate to structure. LC IM-MS was performed on an Agilent 6560 Q-TOF using a C18 reverse-phase method examining both total lipid extracts and lipid classes (free fatty acids, phospholipids (PLs), neutral lipids) following solid phase extraction. Firstly, we investigated how obesity remodels the composition of cell membranes in white adipose tissue, examining both diet (high fat diet; HFD) and genetic (the leptin deficient ob/ob mouse) induced models of obesity. Using a combination of samples and standards, and an in-house database of mass-to-charge (m/z) ratios, retention times, MS/MS spectra, and CCS values of phospholipid and fatty acid standards we confirmed previous reports that both the head group and degree of unsaturation alter CCS, with CCS values increasing with the degree of saturation. Multivariate statistics discriminated adipose tissue from control and ob/ob mice on a regular chow diet as well as both mouse genotypes on HFD. Both HFD and genetic obesity were characterised by a reduction in the diversity of PLs species detected. The lipidome of blood plasma from inflammatory bowel disease (IBD) patients has also been investigated, with IM identifying species associated with arachidonic acid metabolism altered in IBD.

O-444	Identification and discrimination of sparse networks in metabolic systems

PRESENTING AUTHOR: *Lindy Rae, The University of New South Wals, Australia*

CO-AUTHORS: *Benjamin Rowlands, Ben Cassidy*

Identification and comparison of networks in metabolic data can yield valuable systems biochemistry information, allowing use of systems information to identify metabolic compartments, points of deregulation and metabolic patterns associated with disease. Here, we adapted methods for network identification for application to brain metabolism. We used steady-state data sets from guinea pig cortical brain tissue slices incubated with [1-13C]glucose and [1,2-13C]acetate. Acetate preferentially labels glial cells due to silencing of the metabolizing enzyme by acetylation in other brain compartments. Using substrates labelled in this fashion allows us to determine not only the fates of these labels, but also the substrate of origin, since the double or single label result in distinct labelling patterns. The resultant labelling patterns and also total metabolite pools can be subsequently measured using 1H/13C NMR spectroscopy. We constructed conditional independence networks where the direct strength of association between each pair of metabolites is estimated using partial correlation. A standard partial correlation suffers from errors induced by the larger number of interactions to estimate from relatively few data samples. Here, we estimated partial correlations using the MISTIC algorithm [1] which regularizes the calculation by imposing a strict sparsity penalty on the estimated partial correlations, setting many to zero. This, in our relatively simple system produced an interpretable metabolic network, providing insights into the compartmentation of acetate metabolism in brain. Marjanovic, G. et al. Large scale l0 sparse inverse covariance estimation. 2016 IEEE ICASSP

O-445	Towards accessible, standard and reproducible Metabolomics

PRESENTING AUTHOR: *Reza Salek, EMBL-EBI, United Kingdom*

CO-AUTHORS: *MetaboLights Team, PhenoMeNal Team, Christoph Steinbeck, Claire O'Donovan*

Reproducing results in any science are quite challenging as a recent 2016 survey by Nature (http://www.nature.com/news/reality-check-on-reproducibility-1.19961) has shown 2/3 of researchers are concerned about science reproducibility. In the field of metabolomics, for results to become reproducible, descriptions of an investigation in a manuscript are insufficient. To overcome this, and increase the chance of result reproducibility, standard frameworks for data sharing and sharing of experimental data are invaluable. In this presentation, developments in data standards initiatives in metabolomics, including for NMR raw data (COSMOS initiative- http://www.cosmos-fp7.eu/) developments for metabolite identification and for data quality (both joint efforts by MSI and HUPO-PSI) will be discussed. It will also be shown how emerging metabolomics data sharing platforms can promote , accessible data sharing standards. Finally, our own experiences, as well as community efforts in creating metabolomics data analysis workflows, particularly in Galaxy environments which can capture study-specific experimental parameters will be presented. Such workflows would ideally run on a dedicated e-infrastructure platform, such as the ones developed by the PhenoMeNal consortium (http://phenomenal-h2020.eu/home/) on a High Performance Computing (HPC) environment. Such efforts, coupled with wider community involvement can pave the way for a greater standard and reproducible results in data analysis, data integration and reuse of data in metabolomics.

O-453	Advancing Metabolomics through Imaging Mass Spectrometry and Direct Tissue Analysis

PRESENTING AUTHOR: *Richard Yost, University of Florida - Southeast Center for Integrated Metabolomics, United States*

CO-AUTHORS:

Mass spectrometric imaging (MSI) provides a level of chemical and metabolomic information unmatched by any other imaging modality (including histopathology, MRI, and PET scans). Furthermore, MSI offers the potential for rapid and direct analysis of tissue even when an image is not of interest. This presentation will explore innovations in MSI and direct tissue analysis, focusing on sampling methods (including matrix-assisted laser desorption ionization MALDI and real-time in situ microextraction using the flowprobe) along with strategies for increasing the speed, spatial resolution, information content, and quantitative performance of the methods. MSI takes advantage of the remarkable sensitivity and selectivity of mass spectrometry (including high revolution MS and tandem mass spec MS/MS and MSn). Furthermore, MSI can yield insight into hundreds of analytes in a single metabolomics analysis, without labeling. Even when a chemical "image" is not of interest, MSI techniques can provide rapid and direct analysis of tissue, including samples too small for classic GC/MS or LC/MS metabolomic analysis. This presentation will present data for a variety of metabolomics applications, focusing on characterization and biomarker detection in a variety of diseases. These studies will include the potential for rapid screening for skin cancer (melanoma), assessment of liver allographs for liver transplantation, and investigation of treatment modalities in diseases such as Parkinson's.

O-462	Big fat back-track: mapping desaturase activity using ozone-induced dissociation mass spectrometry

PRESENTING AUTHOR: *Stephen Blanksby, Queensland University of Technology, Australia*

CO-AUTHORS: *Reuben Young, Berwyck Poad, David Marshall, Martin Sadowski*

Mass spectrometry is unarguably the pre-eminent technology for the detection, identification and quantification of lipids in biology. Contemporary approaches based on electrospray ionization mass spectrometry can yield a detailed profile of the lipids present within a cell, tissue or organism. While changes in such a profile can be sensitively detected and used as markers of biochemical change, mapping these changes to known biochemical pathways can be hindered by a lack of lipid structural information. Moreover, mass spectrometry – even at very high resolution – can be blind to dynamic processes in lipid metabolism that are not manifested as a change in mass. The most striking example of this phenomenon is the profile of lipid unsaturation. While different levels of unsaturation result in clear mass shifts (i.e., 2 Da for each double bond), changes in the site(s) of unsaturation result in isomeric lipids that have identical mass. We have developed ozone-induced dissociation (OzID) mass spectrometry specifically to visualize the unsaturation profile of lipid extracts. This technology uses the reaction of ionized lipids with ozone inside the mass spectrometer to uniquely assign the site(s) of unsaturation in lipids and reveal the presence of isomers. Application of OzID to a series of prostate cancer cell lines reveals an unusual pattern of unsaturation that can be traced back to the aberrant activity of the FADS2 desaturase enzyme. Tracking carbon-carbon double bond positions through a wide array of lipid classes provides novel insights into enzyme-substrate interactions and allows direct comparison with the available transcript data.

O-473	Towards reliable lipoprotein particle predictions from NMR spectra of human blood: Interlaboratory ring test validation of a rigorously standardized NMR protocol

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CO-AUTHORS: *Sandra Centelles, Huub Hoefsloot, Parvaneh Ebrahimi, Mads Lind, Mette Kristensen, Niels de Roo, Doris Jacobs, John van Duynhoven, Claire Cannet, Fang Fang, Eberhard Humpfer, Hartmut Schäfer, Manfred Spraul, Søren Engelsen, Age Smilde*

Lipoprotein profiling of human blood by 1H Nuclear Magnetic Resonance (NMR) spectroscopy is a rapid and promising approach to monitor health and disease states in medicine and nutrition. However, lack of standardization of measurement protocols has prevented the use of NMR based lipoprotein profiling in meta-studies. In this study, an extremely standardized NMR measurement protocol was applied in a ring test performed across three different laboratories in Europe on plasma and serum samples from 28 individuals. Data was evaluated in terms of (i) spectral differences, (ii) differences in LPD predictions obtained using an existing prediction model and (iii) agreement of predictions with cholesterol concentrations in high and low density lipoproteins (HDL and LDL) particles measured by standardized clinical assays. ANOVA-simultaneous component analysis (ASCA) of the ring test spectral ensemble that contains methylene and methyl peaks (1.4-0.6 ppm) showed that 97.99% of the variance in the data is related to subject, 1.62% to sample type (serum or plasma) and 0.39% to laboratory. This inter-lab variation is in fact smaller than the maximum acceptable intra-lab variation on quality control samples. It is also shown that the reproducibility between laboratories is good enough for the LPD predictions to be exchangeable when the strict conditions for NMR acquisition are maintained. With this validated protocol resulting in highly reproducible prediction of lipoprotein distributions across laboratories, a step is taken towards bringing NMR more into scope of prognostic and diagnostic biomarkers, reducing the need for tedious methods such as ultra-centrifugation or HPLC.

O-479	3D Molecular Cartography of the Exposome and Metabolome of Fruits

PRESENTING AUTHOR: *Louis-Felix Nothias, CNRS / UC San Diego, France*

CO-AUTHORS: *Alexander Aksenov, Alexey Melnik, Ivan Protsyuk, Theodore Alexandrov, David Touboul, Pieter Dorrestein*

The "3D-Plant2Cells" project aims to investigate the impact of pesticides on common fruits metabolome and the microbiota in three dimensions. To achieve that objective, we first developed a mass spectrometry (MS)-based methodology for the 3D molecular cartography of pesticides/metabolites on a fruit surface. For that, we employed cotton swabs to sample locations of common fruits. The corresponding samples were then extracted and analyzed by gas or liquid phase chromatography coupled to MS in both untargeted and targeted modes. The MS data were mapped on a 3D model of corresponding fruit using the 'ili web-based software (Protsyuk et al, 2017), allowing the visualization and sharing of pesticide/metabolite distribution on the fruit surface. Detected pesticides were annotated with GNPS spectral libraries and unknown features annotated with Sirius. The 3D molecular cartography offered unique capabilities for fruit exposomics and metabolomics, as the molecular complexity can be described and visually represented in an easily interpretable topographical molecular maps. The method also capitalizes on combination of targeted mode that offers sensitivity with downstream spectral annotation capability of untargeted MS/MS. To validate the method, we investigated the performance of this swab-based protocol by looking at various aspects of the sampling process including (1) the sensitivity, (2) the compound recovery, (3) the sampling reproducibility, and (4) the influence of the matrix effects related to swab and fruit. We will present methodological developments regarding the validation of the method for the detection and quantification of pesticides, along with the first 3D molecular model of pesticides on common fruits.

O-502	Lighting up the world of metabolomics

PRESENTING AUTHOR: *Roy Goodacre, University of Manchester, United Kingdom*

CO-AUTHORS: *Omar Alharbi, Malama Chisanga, Heidi Fisk, Kat Hollywood, Howbeer Muhamadali, Ali Subaihi, Chloe Westley*

Many different physicochemical techniques are used for the identification and quantification of metabolites. Whilst our group (www.biospec.net) has developed a range of MS-based techniques for metabolomics, and used these for large-scale molecular phenotyping, this presentation will concentrate on our recent advances in Raman spectroscopy. Raman spectroscopy is a non-destructive vibrational spectroscopy technique, and as is well known to most spectroscopists Raman spectroscopy has a rich history for the analysis of chemical species. However, whilst this inelastic light scattering approach 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 analyte(s) with a metal surface (roughed at the nano-scale and hence why we tend to use colloids) 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. In this presentation we shall demonstrate this for the absolute quantification of drugs and metabolites directly in human body fluids. When the sample matrix is complex then prior separation is needed and so we shall also highlight our work in coupling liquid chromatography to Raman. Finally, as Raman has a spatial resolution of ca. 1 um it can be used for imaging of biological systems and this will be demonstrated for drug detection in eukaryotic cells as well as metabolic labeling of bacteria with stable isotopes as a step towards understanding microbial communities.

O-517	Novel methods for data generation and data integration in metabolomics

PRESENTING AUTHOR: *Johan Trygg, Umeå University, Sweden*

CO-AUTHORS:

Today, massive amounts of data experimental data is being generated by modern high-throughput 'omics and sensor technologies with increasing availability and decreasing experimental costs. This overwhelming size and complexity of modern 'omics' and phenotypic data have driven systems analysis towards the adoption of multivariate analysis and network modeling methods. In addition, machine learning methods capability to analyze so called unstructured data (text, images, audio), accelerated by the Python and R open source communities has allowed us to tap into an exciting dimension of so called big data that we can start to integrate with existing structured data (omics, spectroscopy, chemistry, etc…). The principle, garbage in – garbage out, highlights the importance of good quality data. Here, Design of Experiments, DOE, provides easy to use strategies and methods to understand the influence and causality of all the relevant factors and parameters studied. I will share some new developments within DOE that enable the generation of high-quality and representative data in 'omics. In particular, I will focus on generalized fractional factorial designs that allows generation of representative and balanced data, the optimization of data processing operations and balanced and representative selection of subsets from biobanks and larger cohorts. For data integration, I will describe the latest developments of OnPLS, a multi-block analysis technology for statistical integration of complex multi-omics datasets.

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P-1	NormalizeMets: statistical tools for implementing, assessing and choosing normalization methods for metabolomics data

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In the statistical analysis of metabolomics data, normalization is a crucial step, which is necessary for dealing with inevitable sources of unwanted variation such as those originating from multiple batches, laboratories, long runs of samples, and other confounding biological variation. Metabolomics normalization is often considered a grey area where there is a distinct need to develop a greater understanding of when, why, and how to normalize the data. We present a joint graphical user interface within Microsoft Excel and R software as a potential "one-stop shop" for normalizing metabolomics data. The package includes widely-used traditional and recently developed metabolomics normalization methods to (a) remove the unwanted variation component to obtain a normalized data matrix that is suitable for downstream statistical analysis, and/or to (b) accommodate the unwanted variation component in a suitable statistical model designed to answer the research question of interest. The software allows easy comparisons to be made between different normalization methods using several statistical criteria, hence guiding metabolomics researchers to assess and choose a suitable normalization method for a given dataset and a research question. Having chosen the appropriate normalization method, the package can then be used to obtain end statistical outputs for (i) clustering, (ii) classification, (iii) biomarker identification adjusting for confounding factors and (iv) correlation analysis.

P-2	Automated Kits and Devices for Quantitative Metabolomics

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Automation nuclear magnetic spectroscopy (NMR) and mass spectrometry (MS) techniques remains a pressing challenge in the field of metabolomics. Both are powerful techniques, but limited by requirements for highly qualified personnel to operate the equipment and perform time-consuming spectral profiling and data analysis. In an effort to "democratize" metabolomics, The Metabolomics Innovation Centre (TMIC) is developing easy-to-use kits and devices that streamline metabolomic workflows, improve inter-lab reproducibility, increase throughput and reduce costs. The goal is to provide everything necessary to run a metabolomic analysis, except instrumentation. TMIC's NMR kit contains all the components required for 1H NMR analysis, including buffer solution, deuterated internal standards and detailed instructions. The kit also contains an access code for BAYESIL, a web-based, automated NMR spectral profiling server. BAYESIL automatically determines the concentration of NMR-detectable metabolites accurately (~95% correct identification; ~10% quantification error) in < 3 minutes per sample. This NMR kit is compatible with serum, plasma, cerebrospinal fluid, ruminal fluid, milk and fecal water samples. TMIC's GC-MS kit includes a derivatization reagent, internal and alkane standards (C8-C20 and C22-C40), and detailed instructions. The kit contains an access code for GC-AutoFit, an automated, web-based tool, which can identify and quantify up to 120 compounds in urine, serum, plasma, CSF and milk. TMIC is also developing low-cost, portable, multiplexed lateral flow devices, enzyme-based colorimetric assays and electronic impedance sensing systems that are capable of simultaneously measuring multiple metabolites. These kits and devices will enable a wealth of new metabolomics applications in both research and clinical settings.

P-3	Contaminant DB: the Environmental Contaminant Database

PRESENTING AUTHOR: *Naama Karu, Department of Biological Sciences, University of Alberta, Canada*

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ContaminantDB is the world's largest electronic repository containing detailed information on small molecule (<1500 Da) chemical contaminants which can appear in water, air, soil, food, buildings etc. at unnatural levels. ContaminantDB has been designed and developed to facilitate exposome and environmental metabolomics research, and help with the identification of trace compounds / contaminants in humans, animals and plants. Currently ContaminantDB has over 70,000 compounds, 20,000 of these compounds not found in any other chemical database. Every entry contains a validated chemical structure, a description, physico-chemical data, estimated production volume, predicted or known MS/MS spectra and a detailed chemical taxonomy/ontology. Most contaminant entries are classified according to their environmental source, application and potential biological impact (e.g. carcinogens; endocrine-disrupting). Examples for contaminant groups include high-production volume (HPV) chemicals, plasticizers, combustion byproducts, pesticides, pharmaceuticals, wastewater compounds and disinfection byproducts. ContaminantDB was constructed using the same principles as large databases previously developed at Wishart lab: the Human Metabolome Database (HMDB), DrugBank, FoodDB, and the toxic exposome database (T3DB). Many compounds in ContaminantDB are interlinked to these databases as well as external resources. Where data is available, the concentration of the compound in specific matrices is presented, to allow meta-analysis between quantitative studies. Additionally, the database supports various browsing and searching capabilities including structure, text, mass spectra and NMR spectra searches. We believe ContaminantDB will be an important resource for environmental and biological research. Over the coming years, ContaminantDB will be continuously growing in functionality and content to meet the increasing demand from the scientific community.

P-4	A Novel Labeled Metabolomics Workflow applying Isotope Ratio Outlier Analysis (IROA) and SWATH® Acquisition for Unambiguous Compound Identification

PRESENTING AUTHOR: *Chris Hodgkins, SCIEX, Australia*

CO-AUTHORS: *Chris Beecher, Felice de Jong, Baljit Ubhi*

Data dependent, mass spectrometry workflows tend to be the choice for the untargeted metabolomics studies. Data independent techniques such as SWATH® Acquisition are different in that they allow for unbiased data collection and MSMS of every single mass precursor can be collected allowing for information rich datasets. However, unambiguous metabolite identification can be increasingly challenging due to the lack of databases, chemical noise and isobaric compounds. The SWATH analysis of the Isotope Ratio Outlier Analysis (IROA) labeled Internal Standard (IS) provides the first mechanism for simultaneous and unambiguous compound identification and quantitation for unbiased metabolomics analysis. Applying Variable Window SWATH® Acquisition strategy to an IROA Internal Standard (IS) spiked sample made it possible to unambiguously identify and accurately quantify hundreds of biochemicals in a single unbiased metabolomics analysis using a 6600 TripleTOF® high resolution mass spectrometer. Here, we present SWATH-IROA whereby uniquely-labeled IROA metabolites were captured within discrete SWATH windows, and subjected to fragmentation. IROA fragments and adducts were shown to have the identical labeling patterns of their precursor ions, with defined formulae. All artefactual (non-IROA) peaks from the SWATH windows were eliminated and data was quantitated based on MSMS peaks. The combination of IROA and SWATH allows a path in which a basic metabolomic-style system may be used for the accurate quantitation of several hundred compounds in a single sample without the need for a baseline separation. Specific software was developed to automatically find, quantitate and identify all natural abundance peaks that corresponded to their known IROA isotopomers.

P-5	Development of anionic metabolome analysis method using capillary ion chromatography-mass spectrometry

PRESENTING AUTHOR: *Akiyoshi Hirayama, Keio University, Japan*

CO-AUTHORS: *Masaru Tomita, Tomoyoshi Soga*

[Introduction] MS-based platforms (such as GC/MS, LC-MS and CE-MS) have been frequently used in the field of metabolomics. Recently, ion chromatography (IC) coupled with MS has been applied in metabolomics as it was found to be an excellent platform for separation of charged compounds. In this study, we have demonstrated the applicability of IC-MS for anionic metabolome analysis. [Methods] Capillary IC-MS analysis were performed using a Dionex ICS-5000+ system equipped with a Q Exactive Orbitrap MS system (Thermo Fisher Scientific, San Jose, CA) via a ESI probe. An Agilent 1100 series capillary HPLC pump (Agilent Technologies, Waldbronn, Germany) was used to deliver sheath liquid. Anionic metabolites were separated on a Dionex IonPac AS11-HC-4 μm (0.4 × 250 mm, 4 μm; Thermo Fisher Scientific) that was maintained at 35°C. [Preliminary results] Firstly, influence of sheath liquid conditions on IC-MS-based metabolome analysis were investigated, and IPA with 0.1% acetic acid was selected as optimum solution. Under this optimized condition, 49 anionic metabolites, including organic acids, sugar phosphates, nucleotides and coenzymes, were successfully separated and detected with mass spectrometer. Acceptable method validation results were obtained related to reproducibility, linearity and sensitivity of the IC-MS method. Notably, the concentration detection limits of the tested compounds were between 1 and 10 nmol/L despite small volume injection (0.4 ?L). Currently, we are applying this platform to the anionic metabolomic profiling for several cancer cell lines. [Novel aspects] The developed IC-MS platform could be a powerful new tool for anionic metabolome analysis.

P-6	Metabolomic imaging of colorectal tumors in mice and humans

PRESENTING AUTHOR: *Shuichi Shimma, Osaka University, Japan*

CO-AUTHORS: *Yasushi Kojima, Masahiro Aoki, Emi Takeo, Tomoyoshi Soga*

Conventional mass spectrometric analysis using tissue extraction precludes determining the spatial distribution of the metabolites, whereas imaging mass spectrometry (IMS) is a feasible technique to visualize them. However, there are a lot of challenges in performing IMS successfully, especially in sample preparation, making the methodological optimizations essential. We present here optimized sample preparations for mouse and human intestinal tumor tissues. Tissue sections of intestinal tumors of Apc mice and clinical samples of colon cancer were prepared at 8 μm thickness. The obtained sections were thaw-mounted on ITO glass slides (Matsunami glass, Osaka, Japan). To provide tiny crystal matrix seeds, we performed a vacuum vapor deposition of 9-aminoacridine (9-AA) for negative ion detection and ?-cyano-4-hydroxycinnamic acid (CHCA) for positive ion detection using iMLayer (Shimadzu, Kyoto, Japan). After the deposition process, recrystallization was performed using 5% methanol vapor for negative ion and 100-μL CHCA solution spraying (8 mg/mL in 50% methanol/0.1% formic acid) with an artistic air-brush for positive ion. IMS was performed using iMScope (Shimadzu, Kyoto, Japan). In negative ion detection, recrystallization contributed to detect peaks derived from metabolites with high intensity, and enabled simultaneous metabolite detection including sugar phosphates In positive ion detection mode, S-adenosylmethionine (SAM) and S-adenosylhomocysteine (SAH) were clearly detected. Specifically, SAM was accumulated inside the tumor regions only, while SAH showed an even distribution in both the tumor and normal regions of the same sample tissues. Notably, we found that SAM provided ring-like distributions, and that the center of the rings was necrotic.

P-7	Next generation lipid profiling of human red blood cells

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Lipids play important roles in cellular signalling in health and disease. Red blood cells (RBC) are widely studied as carriers of possible lipid biomarkers in clinical studies. For example, the RBC ?-3 index has been used as a predictor of cardiovascular disease. The complexity of the phospholipidome is often underestimated by conventional mass spectrometry. A comprehensive yet unambiguous structural identification of RBC lipids is an integral part of understanding their role in cellular functions. Recently, Pham et al. (2014) reported a novel top-down workflow combining collision- and ozone-induced dissociation (CID/OzID) to yield information rich spectra used to uniquely assign sn-substitution and carbon-carbon double bond position in phospholipids. In complex extracts however, the spectral interpretation was confounded by the presence of isobaric lipids resulting from direct infusion protocols. Chromatographic separation of lipids is one potential solution to this spectral complexity. Following extensive optimization for optimal ozone reaction times and sufficient data points across the chromatographic peak, a successful composite workflow combining two new CID/OzID experimental designs, coupled with reverse-phase liquid chromatography (RPLC) separation using a C30 column, enhanced structural characterization of RBC lipids. We present for the first time a software-plug-in for automating OzID-mass spectral annotations. Infusion and LC strategies were found to be complementary and when examined together, provided a richer picture of the structural diversity of the RBC phospholipidome with preliminary data extending the number of phosphatidylcholines from 30 to almost 100, and provoking interesting questions as to the functional role of this diversity.

P-8	Development of high-sensitive and high-throughput methods for chiral amino acid analysis

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CO-AUTHORS: *Yutaka Konya, Moyu Yaniguchi, Eiichiro Fukusaki*

Amino acids are ubiquitous compounds of significant importance in life science due to their diverse vital functions in living organisms. In ?-amino acids, due to the configuration, the structure is able to form enantiomers. Generally, in order to distinguish these structures, they are commonly referred to as L- or D-amino acid. While L-amino acids obviously predominate in nature, D-amino acids were considered to have relatively minor functions in biological process. However, recent technological advances in separation techniques have promoted studies of chiral amino acids and the distribution of free D-amino acids has been of considerable interest in various fields. A number of analytical methods of amino acid enantioseparation for qualification and quantification have been reported. However, the previous work have never fulfilled both resolution and throughput. Recently, we have demonstrated that eighteen chiral proteinogenic amino acids except proline are perfectly separated by using liquid chromatography-time of flight mass spectrometry (LC-TOFMS) equipped with a chiral crown ether column. This method enabled the high-throughput baseline enantioseparation of amino acids while maintaining excellent peak resolution. However, higher sensitivity and wider dynamic range for quantification would be required in some occasions. Most recently, we successfully constructed a simultaneous analytical system for the enantioseparation of amino acids using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Compared to the LC-TOFMS method, the LC-MS/MS method demonstrated the higher sensitivity required for detection of trace D-amino acids in samples. These analytical methods can be applied to various fields and push D-amino acid research to a new stage.

P-9	Shotgun Lipidomics of prostate cancer cells using ESI-MS Shimadzu 8050 and simplified data analysis by SimLipid software

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Lipidomics is a relatively recent omics field of research which includes complex lipidome analysis. It is an emerging field in biomedical research as lipids play an important role in cell, tissue and organ physiology and have potential as biomarkers of disease or treatment success. Shotgun lipidomics involves identification and quantification of lipids by direct infusion of complex lipid samples into the mass spectrometer without any chromatographic separation. In this study we performed shotgun lipidomics on lipid extracts from prostate cancer cells by electrospray-ionisation triple quadrupole mass spectrometry (Shimadzu 8050). Triple quadrupole functionality was used to perform specific precursor ion (PI) and neutral loss (NL) scans for the identification and relative quantitation of Phospholipids and Glycerolipids; Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), and Phophatidylserine (PS) and Triacylglycerides (TAGs) were performed on lipid extracts from prostate cancer cells. Different instrument parameters including sample infusion rate, collision cell settings, quadrupole scanning rates and resolution were optimised before acquiring sample data. A complex data was generated requiring special software tools for the analysis. Unlike genomics and proteomics, there are not many software tools available for comprehensive data analysis. Hence, we have developed new modules of SimLipid software that enable comprehensive lipidome data analysis using multiplexed PI/NL scans data, remove isotopic overlapping of peaks from multiple spectra in batch mode. The acquired data was directly imported into SimLipid 5.60, and phospholipids and TAGs from different samples were identified and quantified. We will present results containing identification and relative quantification of above lipids in prostate cancer cells.

P-10	Microflow LC-MS/MS Workflow Allows High Sensitivity for Targeted Metabolomics

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Identifying metabolites from urine and plasma are essential to understanding diseases and developing novel therapeutics. Liquid chromatography–tandem mass spectrometry (LC-MS/MS) analysis has become an essential tool for the identification and quantification of metabolites in complex matrices. Here, we describe a robust and sensitive workflow using a M3 MicroLC coupled to a QTRAP® 6500+ mass spectrometer for qualitative and quantitative analysis of polar metabolites. We have implemented a HILIC microflow LC-MS/MS method for profiling polar metabolites using selected reaction monitoring (SRM) and positive/negative polarity switching. Microflow LC-MS/MS, despite its inherent sensitivity advantage, has not been used extensively in metabolomics due to requiring proper sample reconstitution for optimal microLC-hydrophilic interaction. Using this method, we have identified 255 unique metabolites of 310 measured targets (379 Q1/Q3 transitions) with a signal to noise ratio of 10 or greater from plasma/urine, covering metabolites from all major metabolic pathways. Most of the LC-MS/MS methods for targeted metabolomics use traditional analytical flowrates of 350-500 ul/ min and multiple LC-MS/MS or scheduled SRM acquisitions to profile such high number of metabolites as compared to our single 30 min workflow. This workflow could be used for relative quantification, and integrated peak areas can be used for quantitation and metabolic pathway analyses across different samples. The sample preparation takes ~4 h from metabolite extraction to peak integration and data analysis.

P-11	NON-UNIFORM DISTRIBUTION OF METABOLITES IN ROOTS AS A RESULT OF A SHORT TERM SALINITY STRESS IN DIFFERING BARLEY CULTIVARS

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Soil salinity adversely affects agriculture by inhibiting plant growth and reducing yield. Barley is rated as salt-tolerant among cereal crops and exhibits a great variation in salt tolerance amongst its cultivars. Changes in the total root lipid and spatial metabolite profile were observed in salt-treated barley roots using metabolite profiling techniques. Matrix Assisted Laser Desorption Ionisation Mass Spectrometry Imaging (MALDI-MSI) was employed to examine plant metabolism. This project aims to analyse the differences in the spatial distribution metabolites in developing barley roots grown under control and saline conditions of two feed (Hindmarsh, Mundah) and two malting (Gairdner and Clipper) cultivars to enhance our understanding of potential salinity tolerance mechanisms. Barley seeds were germinated, grown on agar plates and subjected to a short term 150 mM NaCl stress. Roots were analysed using a Bruker SolariX XR-FT-ICR-MS and a Sciex 6600 TripleTOF-MS, for spatial and untargeted lipidomics, respectively. The combination of LC-MS and MSI identified a large number of metabolites and lipids with a unique spatial distribution. MSI was capable of discriminating salt vs control treated roots, identifying major lipid changes under salinity in a spatial manner. Non-uniform spatial distribution of metabolites was observed among barley cultivars. Major PC lipid species and oligosaccharides were identified to change the most in salt-treated roots compared to control. This work helped to uncover metabolic biology that hasn't been possible using traditional metabolomics methodology.

P-13	Development of high sensitivity CE-MS metabolome analysis system using high resolusion mass spectrometer

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Capillary electrophoresis mass spectrometer (CE-MS) has been widely applied in metabolomics. High resolution mass spectrometer (HRMS) has not been yet up to fully fledged application for CE-MS in spite of the recent advance of HRMS. Electrospray ionization (ESI) source with grounded nebulizer is suited for CE-MS but a number of mass spectrometer manufacturer employ ESI source with high voltage applied nebulizer. In this study, we developed a novel ESI source adapter that converted from high voltage applied nebulizer to grounded nebulizer. We performed metabolome analysis by using high sensitive CE-HRMS system that combined CE with HRMS using the developed ESI source adapter. We targeted primary metabolites (amino acids, peptides, nucleobases, organic acids, phosphate compounds and other ionic metabolites). Our developed system of CE-HRMS was able to improve 10-fold in detection sensitivity compared with capillary electrophoresis time of flight mass spectrometer (CE-TOFMS). Calibration curves of a number of primary metabolites showed three to four digits quantitative dynamic range. In body fluid sample, we demonstrated that more than twice the number of metabolites were detected using the developed system relative to using CE-TOFMS. The developed system is expected to become an effective tool for biomarker discovery.

P-14	Untargeted stable isotope tracing (USIT) reveals rewiring of glutamate anaplerosis as a common metabolic adaptation in diseases arising from mitochondrial DNA mutations

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USIT is a novel in-house untargeted stable isotope tracing workflow that informs on: (1) all features that incorporate isotope; (2) the number of incorporated isotope atoms; (3) fractional incorporation of isotope atoms; (4) total incorporated isotope and, (5) effects of cell treatments on 1-4. In USIT, we first find differentially-expressed features by comparing heavy vs light-isotope treated cells, then use these features as targets to generate curated isotopologues expected for heavy isotope incorporation. Apparent incorporation is confirmed by a reciprocal shift in isotopologue spectral patterns (i.e., heavy isotopolgue increases with a reciprocal decrease in light isotopologue fractional abundance). Using USIT, we identified a previously unappreciated glutamine-derived ?-ketoglutarate (?KG) oxidative flux concomitant with cataplerotic efflux of aspartate and subsequent cytosolic conversion to lactate and alanine in human mitochondrial DNA (mtDNA) mutant cells harboring disease-associated partial oxidative phosphorylation defects. This novel pathway maintains a continuous glutamine influx into the TCA cycle via a mitochondrial glutamate/aspartate antiporter, sustains substrate-level ATP production, contributes to re-oxidization of glycolytic NADH to NAD and provides a source of cytosolic NADPH for redox maintenance. The current finding contrasts with the predominant reductive carboxylation of ?KG previously observed in engineered cell culture models that exhibit a complete loss of oxidative phosphorylation, a lethal condition never seen in patients. Notably, stimulating the oxidative flux with ?KG supplementation enhanced the viability of diverse human mtDNA mutant cell lines grown under obligatory oxidative conditions, which are otherwise lethal. These findings establish a rationale for dietary supplementation with?KG in mitochondrial diseases.

P-15	Developing an open learning environment unit on metabolomics

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The advent in recent years of hardware and software that facilitates the automatic collection and analysis of metabolomics data has resulted in a proliferation of life scientists, clinical and veterinary researchers, and their students undertaking metabolomics studies. To support these researchers, the development of freely available resources and courses to assist with study design, data acquisition, processing and/or analysis is highly desirable. Given the diverse backgrounds of these researchers, from undergraduates to experts in their respective fields, the Open Learning Environment (OLE) provides a very suitable platform for delivery of metabolomics resources. OLE has been hailed as a flexible and highly effective approach for the provision of novel and cross-disciplinary skillsets to students with varying amounts of prior knowledge and from different educational backgrounds. We are currently developing an OLE unit with learning modules that focus on key aspects of metabolomics, from study design, data processing and analytics to linking the findings with molecular/cellular data. As part of this undertaking, we have recorded a lecture series on metabolomics that was presented by Prof David Wishart and is scheduled to be released on the ANZMAG Youtube channel in April. This lecture series will be complemented by a set of online tutorials and a discussion forum. We hope that this unit will provide researchers new to the field of metabolomics with a foundation from which they can access other resources, including contacts with fellow researchers and exchange of expertise.

P-16	Tissue-specific sample dilution for reliable untargeted metabolomics

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Untargeted metabolite profiling studies aim to measure as many metabolites as possible. However, no single analytical technique can cover the full metabolome. To date, liquid chromatography-mass spectrometry (LC-MS) offers the most comprehensive metabolite coverage. Although much attention has been paid to optimisation of the extraction solvent for maximum metabolome coverage, one overlooked aspect is the analytical quality of the LC-MS measurements. Matrix effects produced by different tissue types can interfere with ionisation efficiency and different tissue types can have very different metabolite composition and abundance. Thus the composition and concentration of the extract may not always be compatible with the loading capacity of the separation column or the linear dynamic range of the mass spectrometer. In this study, we performed a bi-phasic solvent extraction and evaluated the use of different reconstitution volumes to re-dissolve the lipid and aqueous extracts from muscle, adipose and liver tissues. We demonstrate that the LC-MS signals do not always scale linearly with the analyte concentrations. Our results show that the optimum concentrations of tissue to reconstitution solvent for liver, muscle and adipose lipid fractions were 59.5 mg/ml, 29.7 mg/ml and 5.95 mg/ml respectively and 64.9 mg/ml for aqueous fraction from all three tissues. This study highlights the importance of an evaporation/reconstitution step during the sample preparation and we recommend a serial dilution experiment to determine the optimal reconstitution volume for each specific sample type prior to instrumental analysis.

P-17	Automated System for High Throughput Targeted Metabolomic Analysis

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In the context of industrial synthetic biology, high throughput targeted metabolic analysis of the diverse chemical space of products and pathway intermediates enables rapid cycles of strain optimization to improve or troubleshoot pathway bottlenecks. We have developed a metabolomics pipeline that combines a software tool for Automated Method Prediction (AMP), a high throughput MS system for data acquisition and an automated data processing system. Given a specific set of metabolic targets for each sample, AMP selects the smallest set of analytical methods (from a predefined set of 8) based on physicochemical properties of the targets. The LC-MS system is capable of processing samples at approximately 2 minute per sample and automatically switching between multiple methods via column and solvent selection valving. The current LC-MS method set includes 8 methods. Acquired data is analyzed and scored using an automated process to generate a standardized output which is transferred to a LIMS system for use in evaluating and informing strain optimization processes. The system we have developed represents a significant advance for both increasing throughput of metabolomics measurements and reducing the amount of expert time-intensive work involved in method selection and data processing. The current system was developed for accelerating microbial strain optimization for industrial chemical production but has potential for advancing high throughput metabolomics measurements in other contexts as well.

P-18	Automatic CCS and MS/MS Library Creation and Application for Large Scale Metabolic and Lipidomic Profiling

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Metabolomics and Lipidomics involve identification and quantification of chemical fingerprint of cellular processes within a biological system. Precise identification is a major challenge since polar metabolites and lipids are chemically and structurally diverse and span a wide mass range. Chromatographic separation does not typically resolve all components, and often lacks retention time reproducibility. The addition of ion mobility, a gas phase separation of ion, increases peak capacity, selectivity, and can potentially resolve isomeric/isobaric. Benefits of collision cross-section (CCS) library obtained from mobility drift times together with accurate tandem mass spectrometry data (MS/MS) of human metabolites/lipids, will be shown to be an additional source of reference to aid metabolite and lipid identification. ESI and MALDI-MS were used to measure ion drift-times on Synapt G2-Si and Vion time-of-flight (TOF) high resolution mass spectrometers. IM was used to measure CCS values in both positive and negative ESI modes in three differently laboratories. The preliminary data showed excellent correlation (median centering < 2%). A tandem mass spectrum of each ionised metabolite/lipid was also acquired and added to the scientific database. A sub-set of the CCS values were verified a Vion instrument to show the across instrument transferability of CCS, and on a MALDI-IM-MS to demonstrate the ionization independence of CCS measurements. Utility of CCS library is demonstrated using an analysis of human plasma sample. Spiked metabolite/lipid standards were identified using a combination of accurate mass measurements and CCS values. The addition of CCS measurements improved the confidence in identification compared to traditional analytical approaches

P-19	Speaq2: Large scale NMR metabolomics data analysis made easy

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Introduction Many present day metabolomics experiments rely on both LC-MS and NMR spectrometry data to fully quantify the available information. NMR tools often lack the automation potential of current advanced LC-MS tools as manual intervention is still required in certain steps. This is a consequence of the fact that most workflows rely on interval methods, such as binning/bucketting, to process these spectra. This approach effectively summarizes the spectra and allows easier data processing and statistics. However, several problems are introduced, both in pre-processing and in the subsequent analysis, that compromise the automation potential. What we did We present a new user-friendly workflow for the analysis of NMR spectra that uses wavelets to fully automate the process of converting raw spectra to peaks, which are then aligned and grouped into features. Since wavelets are also often used in peak picking for LC-MS data (to convert spectra into peaks with minimal information loss) this method advances the integration of LC-MS and NMR data. A crucial aspect is that this is not just another NMR data analysis tool but it can be used to improve other tools that rely on the binning method or tools that start with already processed spectra into peak lists. By using the wavelet based method the quality of these peak lists can be improved. The framework is validated by replicating the results of two papers demonstrating the speed, user-friendliness, minimal user interaction and improved results. Can I use it? The algorithms are made available in the speaq2 R-Package.

P-21	A Sparse Partial Least Squares Algorithm Based on Sure Independence Screening Method

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Partial least squares (PLS) regression is a widely-used dimension reduction method in many areas of scientific discoveries. However, it has been shown that the consistency property of the PLS algorithm does not extend to cases with very large number of variables p and small number of samples n. To overcome the issue, sparsity can be imposed to the dimension reduction step of PLS so that the sparse version of PLS (SPLS) algorithm can achieve dimension reduction and variable selection simultaneously. Here, we present a new SPLS method called sure-independence-screening based sparse partial least squares (SIS-SPLS) algorithm. By incorporating the published SIS method, the current SIS-SPLS algorithm is capable of achieving a near ideal selection of all important variables. In addition, we also provide an implementation of SIS-SPLS by using extended Bayesian information criterion (BIC) . This criterion is more flexible as compared to tuning parameters used in published SPLS methods. The developed method was evaluated using a number of numerical studies which involved both simulation and real datasets. The result showed that the proposed SIS-SPLS method offered low mean squared prediction errors. In addition, it was found capable in selecting all important variables and excluding nearly all irrelevant variables in the dimension reduction procedure. The SIS-SPLS algorithm proposed in the current work may serve as an alternative SPLS method for the analysis of modern biological data.

P-22	A Workflow for the Assessment of the quality of Isotopologue Distribution Measurements by Mass Spectrometry

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Fluxomics is the measurement of actual metabolic reaction rates within biological systems (intracellular or intratissular fluxes), and has become a powerful tool for quantifying cell physiology for various applications as metabolic engineering, biotechnology or biomedical research. The most relevant approach to measure fluxes in living cells or tissues is based on isotope labeling strategies (13C) coupled to the detailed analysis of isotope incorporation into metabolites, measured by mass spectrometry or NMR spectroscopy. The quality of the biological insights provided by these approaches strongly depends on the quality of the isotopic measurements. However, despite decades of isotopic studies of metabolism based on mass spectrometry, there is still no method to evaluate the reliability of MS-based isotopic measurements. Here, we present a method to address this issue. In this method a biologically-produced sample containing metabolites with fully predictable isotopologue distributions [1] is produced and used as reference material for evaluation of analytical methods. This work provides a general framework for assessing the quality of MS-based isotopic measurements, as well as for instrument qualification. . In addition, these results have highlight a new method to determine the working range adapted to these measurements. This approach is currently applied to test the analytical capacity of different MS technologies for isotopic studies on the French infrastructure MetaboHUB. [1] Millard, P; Massou, S; Portais, J-C; Letisse, F. Anal. Chem. 2014, 86, 10288-95

P-23	Improving throughput of pre-analytical sample handling for NMR-based serum metabolomics

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Mass-spectrometry (MS) and nuclear magnetic resonance (NMR) spectroscopy are the two most widely used analytical techniques to analyze blood metabolites. The main advantage with NMR is the possibility to identify and with high precision determine the concentration of individual metabolites also in complex mixtures, combined with a very high degree of reproducibility. The latter fact makes it attractive for use in studies of large numbers of samples, but also for long-term studies or continuous series of samples. NMR is less sensitive and typically 30-50 metabolites can routinely be determined in a standard targeted approach (1,2). The lab of Daniel Raftery recently presented an approach where a 800 MHz 5 mm cryoprobe and protein precipitation using methanol further extended the number of identifiable metabolites. We have adapted this procedure using state-of-the-art technology (Bruker SamplePro pipetting robots and a Bruker 800 MHz 3mm TCI cryoprobe) in a high-throughput mode which requires decreased serum amounts and offers increased sensitivity. Similarly, the automated butanol-methanol lipid extraction developed by Ståhlman and coworkers has been implemented with an Agilent Bravo robot to enable NMR studies on both lipid-extracted and methanol-precipitated material from a sample volume of 250 µl.

P-24	Recursive weighted Partial Least Squares Discriminant Analysis (rPLS-DA): a new method for finding important metabolite variables in untargeted metabolomics research

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Recursive weighted Partial Least Squares Discriminant Analysis (rPLS-DA) is a novel classification tool which combines the discriminative power of PLS-DA with the innovative variable selection method provided by rPLS. In the recursive weighted version of PLS-DA, the selection of the discriminant variables is made by iteratively re-weighting them in a ridge regression approach using the regression coefficients calculated by the previous (n-1) PLS model. By converging to a few variables, rPLS-DA provides a direct 1:1 metabolite model interpretation. However, it is based on correlation and not causality – it will just find the combination of variables that best separates the classes. The classification performance of rPLS-DA was tested using two large datasets from two different metabolomics investigations. In the first example, the metabolite profiles of a total of 892 milk samples from Danish Holstein-Friesian (n =456) and Jersey (n=436) cows were measured by 1H NMR spectroscopy. PLS-DA was firstly applied with the aim of discriminating the milk samples according to the different genetic backgrounds. The strong overlapping of metabolite markers in the PLS-DA model severely hampered variables identification and model interpretation. In contrast, rPLS-DA resulted in a simpler model with fewer discriminant metabolites contributing the most to samples classification. In the second study, rPLS-DA was applied for investigating the metabolic differences in plasma samples of individuals in a dietary intervention study, highlighting its high potential in dietary biomarkers discovery.

P-25	A Comprehensive Metabolomic Approach for identifying the Enantioeospecific Response of Urea Cycle of Tobacco Cells under Salinity

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Metabolite profiling provides insight into living cells metabolism and behavior. Herein, metabolism of L- and D-ornithine was investigated to ascertain the role of enantiomers on the urea cycle of tobacco cells under normal and stress conditions. A combined GC and LC based metabolite profiling optimized for plant cell culture, with around 100 identified metabolites, indicated a specific up-regulation of carbohydrates, polyamines and organic sulfur (S) related metabolites by D-Orn. LC-ESI/MS based profiling of the amines containing groups metabolites showed an inhibition of the biosynthesis of several amino acids as a result of salinity induced damage in the cells. Additionally, Arg and other urea cycle related metabolites including, proline, putrescine and citrulline showed relevant increases in the D-Orn treated cells. These results indicated that D-Orn, as a D-amino acid (D-AA), can effectively participate in both polyamines and organic S related AAs biosynthesis, e.g. cysteine and methionine, compared to its L-enantiomer counterpart. In parallel, GC/MS based metabolite profiling demonstrated that D-Orn promoted up-regulation monosccharides biosynthesis alleviated tobacco cells. Furthermore, L-Orn had remarkable effects on fatty acids and phenolics content of the cells. Finally, results presented herein demonstrate for the first time that selective regulation of certain metabolic pathways and subsequently cell growth enhancement and stress alleviation can be potentially performed by means of stereoselection of AAs.

P-26	SWATH® Acquisition - a Unique Approach for Untargeted Metabolomics Applications

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SWATH® acquisition, a data independent acquisition (DIA) workflow is well adopted in quantitative discovery proteomics, but not commonly used in discovery metabolomics. SWATH® acquisition combines the benefits of quantitation at the MS2-level of targeted MRM-based workflows with MS2- level based untargeted identification for metabolite identification of DDA workflows with the comprehensive nature of the MSMSall workflow. Because of the comprehensive, non-stochastic nature of the fragmentation in SWATH® acquisition, more fragmentation and thus structural information of the analytes compared to the DDA approach is achievable. Reproducibility and coverage is lower for DDA approaches compared to DIA workflows. Here we describe the improvements in metabolite coverage using SWATH® acquisition without sacrificing quantitation. Results obtained demonstrated a significant improvement of metabolites identified at the MS2 level by using SWATH® with variable windows in comparison with fixed windows in all analyzed matrices. We compared the ID rate from SWATH® acquisition to standard DDA. Here we were able to identify almost twice as many metabolites from the spectral library by SWATH® acquisition using 30 variable windows than by DDA. More confident MS2 based identifications then lead to more quantifiable metabolites in a metabolite expression experiment, which at the end allows better understanding of the biology. Spiked experiments into matrix samples of heavy labeled metabolites highlighted ten times higher sensitivity (signal-to-noise) using the MS2 ion to quantitate versus the traditional MS1 approach thus demonstrating the specificity nature of SWATH acquisition to more traditional data dependent approaches

P-27	Analysis of Deuterium-Labeled Lipids in E. Coli Extracts with High Resolution OrbitrapTM Mass Spectrometry

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Labeling experiments are often used to estimate de novo biosynthesis rates in lipids. In D2O-labeling experiments the quantification of absolute D abundance is complicated by 13C, which occurs naturally. Therefore, it would be beneficial if deuterium incorporation could be differentiated and resolved from the 13C peak. Here we present analysis of deuterium-labeled lipids, extracted from E. coli, by utilizing a reverse-phase chromatography coupled to an Orbitrap TM Fusion TM Lumos TM Tribrid TM mass spectrometer operated at a resolution of 120,000 to 500,000 (FWHM at m/z 200) for the LC-MS/MS scans. Lipid identification was performed with LipidSearchTM 4.1 software. The major lipid sub-classes identified, for E. coli, in negative ion mode were phosphatidylethanolamine, phosphatidylglycerol, and cardiolipin. The M+1 and M+2 isotopes of selected deuterium-labeled cardiolipin, phosphatidylethanolamine and phosphatidylglycerol species were fragmented with HCD. HCD product ions were detected using the Orbitrap mass analyzer at mass resolution of 120K, 240K or 500K. Inspection of the deuterium-labeled lipid MS/MS spectra obtained at 240K or 500K resolution gave 2H containing product ions that were resolved from the 13C product ions. For instance, MS/MS of cardiolipin CL(62:1) [M-H]- anions, at 500K resolution, gave labeled 14:0 glycerol-phosphate product ions that were baseline-resolved including 13C2C15H32O6P (m/z = 365.1998), 13C1C16H31D1O6P (m/z = 365.2027) and C17H30D2O6P (m/z = 365.2057). These species differ by 0.0029 Da and require a resolution of 244K to be resolved. Our data demonstrates that in deuterium-labeling experiments, high resolution at the MS/MS level is essential for resolving the deuterium from the 13C peak.

P-28	Calibration-Curve-Locking database for metabolome analysis by GC/MS

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Calibration-Curve-Locking Databases (CCLD) have been constructed for automatic compound search and semi-quantitative screening by GC/MS in fields of forensic medicine and residual pesticide measuring [1-3]. CCLDs contain the retention time, calibration curve and electron impact ionization mass spectrum obtained under stable apparatus condition. We constructed a novel CCLD for metabolomics study field. All standard compounds and biological samples were subjected to GC-MS just after the derivatization under stable apparatus conditions using following strategies. 1: DFTPP tuning for reproducible and uniform mass spectrum. 2: Retention-Time Locking technique to fix the retention times. 3: Automation of derivatization followed by injection to GC-MS by PAL RTC (CTC Analytics AG). 4: The system performance checking by a criteria sample mix solution. One target (quantifier) ion and one or more qualifier ion were selected for each compound based on the results of standard substances analysis, and a calibration curve was obtained by plotting the peak area ratio of the target compound to the IS versus the amount of target compound. These data were registered as the novel CCLD using MassHunter Quantitative Analysis software (Agilent Technologies), which enables automatic compound search and quantification by target deconvolution and quantification algorithm. We examined the applicability of the constructed database to analyzing serum samples, resulting time- and labor-saving semi-qualitative screening without the need for standard substances. 1) Kadokami, K, et al., J. Chromatogr., A 1089, 219-226 (2005). 2) Ishida, T., et al., Rapid Commun. Mass Spectrom. 21, 3129-3138 (2007). 3) Kudo, K., et al., Leg. Med. 14, 93-100 (2012).

P-29	The Road to Metabolomics Harmonization: Development of NIST "Omics" Reference Materials and Data

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As the field of metabolomics rapidly advances, there has been a parallel expansion of different measurement platforms capable of evaluating a broad range of diverse metabolites. Accordingly, it has become vital for NIST to engage the metabolomics community in measurement harmonization efforts in order to confirm and validate biological discoveries. Building upon lessons learned from community implementation and utilization of the National Institute of Science and Technology (NIST) SRM 1950 – Metabolites in Frozen Human Plasma, NIST is currently developing new reference materials (RMs) that may serve as valuable materials to harmonize metabolomics measurements. Through these endeavors, NIST will be able to provide affordable, stable, homogenous RMs on demand to meet the needs of the metabolomics community. The goal is for these RMs and their associated data (RMDs) to be evaluated by the metabolomics community via an open-ended quality assurance program/interlaboratory comparison infrastructure, which results in evolving, shared data assessments. Specifically, a suite of human urine and plasma RMs is currently being developed. In addition, we are broadening our RM efforts to include dried-blood spots, as dried-blood spots are an emerging biospecimen useful for many metabolomics applications. To complement these efforts, a solution-based RM for use in assessing the relative performance and reproducibility of non-targeted LC-MS/MS metabolomics analytical techniques is also under development. Ultimately, NIST aspires to provide metabolomics laboratories with RMDs complemented by their shared data assessments, relevant method information, essential performance metrics, and overall consensus measurement results to improve interlaboratory comparability of metabolomics measurements.

P-30	Correction of errors from pre-analytical sample management

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Health-related metabolomics studies often require the use of biobanked, or otherwise stored, cohort samples. However, sample handling is complex and many pre-analytical variables, including collection and processing of biological samples, can alter sample integrity. Multiple study centers or the use of legacy samples with limited sample management metadata further adds complexity. Novel means to understand and take into account such variability would enable high-quality research on archived samples. We have investigated alterations in the plasma metabolome, measured by NMR, as a function of pre-centrifugation conditions (1-36 h pre-centrifugation delay time at 4°C and 22°C) in 16 individuals. Using the obtained data, we developed a procedure to predict, model and correct reproducible alterations in the plasma metabolome: Pre-centrifugation temperature and delay times were predicted using random forest modeling and validated on independent samples. Reproducible changes in the metabolome were modeled using a cluster-based approach, revealing effects of delay time on energy metabolism intermediates, especially at 22°C. We also found large, specific variability in metabolite concentrations after 3 h delay at 4°C, predominantly of lipids. Error correction using the cluster-based approach resulted in significant improvement of data quality, particularly at 22°C. Our results suggest potential to decrease the impact of undesired, delay-induced variability and the possibility to predict pre-centrifugation conditions in archived samples before use in costly downstream applications. However, results need to be confirmed in multiple, large sample sets and with analytical techniques offering wider metabolome coverage, such as LC-MS

P-31	Quantification of bioactive N-acylethanolamines in human plasma

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N-acylethanolamines, an endogenous lipid mediator in various animals, is amide compound group with a chemical structure condensed from long chain fatty acid and ethanol amide. In this chemical group, N-arachidonoylethanolamine (anandamide) is arachidonic acid which was discovered as an endogenous ligand of cannabinoid receptor (CB1) and provides cannabinoid-like biological activities, such as analgesia and hypotensive effects. In the same class, N-palmitoylethanolamine provides anti-inflammatory and analgesic action, and N-oleoylethanolamine function as anti-inflammatory. Thus, N-acylethanolamines are considered as potential biomarkers for various diseases. Here, we developed lipid profiling methods using liquid chromatography-tandem mass spectrometry (LC-MS/MS) to profile these metabolites in Tsuruoka metabolomics study, a large prospective cohort study in Japan, and cataloged the concentration of the N-acylethanolamines metabolites. The analytes were puri?ed 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 (Mono-SpinC18, GL Sciences Inc. Tokyo, Japan) to eliminate triacylglyceride (TG) and phospholipid phosphatidylcholine (PC) in the analytes. Totally, 10 kinds of N-acylethanolamines, including anandamide, was quantified in approximately a thousand subjects. The profiled data would contribution to understanding relationship among N-acylethanolamines and some diseases.

P-32	cosmiq - COmbining Single Masses Into Quantities

PRESENTING AUTHOR: *Endre Laczko, Functional Genomics Center Zurich, Switzerland*

CO-AUTHORS: *Endre Laczko, David Fischer*

cosmiq is a Bioconductor package for the preprocessing of liquid- or gas - chromatography mass spectrometry (LCMS/GCMS) data with a focus on metabolomics or lipidomics applications. To improve the detection of low abundant signals, cosmiq generates master maps of the mZ/RT space from all acquired runs before a peak detection algorithm is applied. The result is a more robust identification and quantification of low-intensity MS signals compared to conventional approaches where peak picking is performed in each LCMS/GCMS file separately. The cosmiq package builds on the xcmsSet object structure and can be therefore integrated well with the package xcms as an alternative preprocessing step. The cosmiq algorithm consists of the following steps: Load the mass spectrometric measurement Combine spectra Detect relevant masses Generate and combine extracted ion chromatograms Detect chromatographic peaks Quantification of detected peaks Output the results The usage of the software will be demonstrated on a typical data set. Package Short Url: http://bioconductor.org/packages/cosmiq/

P-33	Microbial metabolites imaging to understand spatial re-distribution and localization of metabolites in single and mixed-species biofilms

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CO-AUTHORS: *Adelicia Y.L.Li, Shimma Shuichi, Sanjay Swarup*

Most microorganisms reside in surface-bound multicellular communities, known as biofilms, working in coherence to maximize the availability of nutrients, defend each other from adverse environmental conditions, and launch coordinated expeditions in search of new territory. Most of these processes among microbial community are carefully mediated and orchestrated by production and mitigation of assortment of metabolites, often creating gradients in micron scales at species boundaries. Current knowledge about metabolites in microbial communities comes largely from sample extraction-based methods, which loses spatial information. Imaging mass spectrometry (IMS) of metabolites coupled with traditional approaches could possibly overcome this limitation and allow spatial insights at community levels. We have been developing MALDI-IMS under microscope using iMScope, wherein images of fluorescence protein tagged cells in biofilms directly grown on cover slips can be overlaid with those from IMS. In the present study, methods for metabolites imaging of single species and mixed species biofilms of Pseudomonas aeruginosa (YFP-tagged) and Klebsiella pneumoniae (DS red-tagged), grown in flow-cell chambers, were optimized. They were harvested and dried before proceeding to application of ionization matrix consisting of 9-aminoacridine (9-AA). We optimized both, sublimation methods using iMLayer (Shimadzu, Japan) and re-crystallization of 9-AA. The prepared biofilm samples were analyzed using iMScope TRIO (Shimadzu, Japan) in the negative ion detection mode. A number of unique mass features were identified that were species-specific, intra- as well as extra- cellularly localized. Interestingly, some unique mixed- species-specific mass features were also identified. Our results indicate MALDI-IMS is a

P-34	IMPLEMENATION OF A NOVEL SCANNING QUADRUPOLE DIA ACQUISITION METHOD FOR DESI IMAGING

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CO-AUTHORS: *Mark Towers, Emrys Jones, Philippa Hart, Emmanuelle Claude, James Langridge*

Here we have assessed the applicability of this new method and optimisation of settings for a DESI imaging analysis. The Sonar method for DESI imaging consisted of two alternating functions. In both cases the quadrupole was scanned multiple times across the mass range with a pre set quadrupole window. In the first function (precursor function) the collision energy was fixed at 6ev, in the second function collision energy was applied to fragment the ions (MS/MS function). The functions alternated between pixels to generate images of precursors and of fragments in a single experiment. The precursor and MS/MS functions were subsequently time aligned to relate the fragments to precursors for identification of multiple species from a single imaging run. Proof of concept experiments have been performed analysing a mouse brain tissue section in negative mode scanning the quadrupole from m/z 750-950 with a quad window of 8 Da. For the MS/MS function the collision energy was fixed at 30eV. Reviewing the data with Driftscope and HDImaging a number of time aligned precursors / fragments could be identified. One example was a PS(18:0_22:6), from which fragments of the neutral losses of serine, the sn1 and sn2 RCOOH groups + serine were observed as well as the sn1 / sn2 RCOO- ions. In addition to the time aligned nature of the precursor/ fragment spectra, the spatial distribution of the precursors and fragments in the imaging data could also be used to further refine the precursor fragment assignments.

P-35	NMR based metabolomics of OCT-embedded frozen kidney samples in mouse and man through a simple and convenient pre-analytical protocol

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Introduction. Pre-analytical processing significantly affects tissue metabolomes. Since most frozen kidney samples are stored after embedding, standardization of cryoprotective medium removal before metabolomics is essential. Objectives. We used rodent and human kidney samples to develop an easy and robust pre-analytical procedure compatible with Nuclear Magnetic Resonance (NMR)-based metabolomics. Methods. In mice, renal ischemia was induced for 30 minutes,followed by 48-hour reperfusion (I/R, n=6). Right kidneys were transversally cut in 2 fragments, and snap-frozen in liquid nitrogen (LN2) or in OCT (Optimal Cutting Temperature) fixative. In man, double kidney biopsies were simultaneously obtained before transplantation (n=15), and snap-frozen in LN2 or OCT. Results. NMR spectrum of pure OCT highlighted 2 major peaks, i.e. from 3.4 to 4.2 ppm and from 1.2 to 2.2 ppm. NMR spectra of mouse OCT kidneys were biased at 3.7. By contrast, NMR analyses of mouse OCT kidneys iteratively rinsed in saline significantly discriminated sham versus I/R groups, with Q^2 at 0.695 (to be compared with Q^2 at 0.866 for LN2 sham versus I/R kidneys). Discriminant metabolites were analogous in both OCT and LN2 kidneys, with a correlation coefficient of 0.83. In man, iteratively rinsing OCT kidneys in saline almost completely eliminated the spectral 3.7-peak, thereby making metabolomes of OCT kidneys interpretable and similar to LN2 samples, with a correlation coefficient of 0.73. Conclusion. NMR metabolomics using OCT-frozen kidney samples is valuable in mouse and man, following standardized OCT removal. This may help use residual biobanked human tissues to better understand renal pathophysiology.

P-36	metaX: a flexible and comprehensive software for processing metabolomics data

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Non-targeted metabolomics based on mass spectrometry enables high-throughput profiling of the metabolites in a biological sample. The large amount of data generated from mass spectrometry requires intensive computational processing for annotation of mass spectra and identification of metabolites. Computational analysis tools that are fully integrated with multiple functions and are easily operated by users who lack extensive knowledge in programing are needed in this research field. We herein developed an R package, metaX, that is capable of end-to-end metabolomics data analysis through a set of interchangeable modules. Specifically, metaX provides several functions, such as peak picking and annotation, data quality assessment, missing value imputation, data normalization, univariate and multivariate statistics, power analysis and sample size estimation, receiver operating characteristic analysis, biomarker selection, pathway annotation, correlation network analysis, and metabolite identification. In addition, metaX offers a web- based interface (http://metax.genomics.cn) for data quality assessment and normalization method evaluation, and it generates an HTML-based report with a visualized interface. The metaX utilities were demonstrated with a published metabolomics dataset on a large scale. The software is available for operation as either a web-based graphical user interface (GUI) or in the form of command line functions. The package and the example reports are available at http://metax.genomics.cn/. The pipeline of metaX is platform-independent and is easy to use for analysis of metabolomics data generated from mass spectrometry.

P-37	AI-based peak detection for mass chromatogram

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CO-AUTHORS: *Atsushi Ogiwara*

Mass spectrometry (MS), including MS combined with gas, liquid, or ion chromatography or capillary electrophoresis, is an essential tool for metabolomics, and it significantly reduces analytical time and effort. However, many researchers in the metabolomics field still aspire to further improvements that can be applied to the vast amount of MS data. Peak detection for MS chromatograms is processed within the MS operating software by computational algorithms with user-defined parameters, but there are no versatile algorithms and parameters for diverse chromatographic shapes. It is, therefore, essential to evaluate peak detection results by visual inspection and to correct the results manually if the detection algorithms do not work properly. This manual effort not only wastes time but can also introduce human error or variation in the criteria for evaluation. Researchers, therefore, require peak detection algorithms that rapidly provide results consistent with those determined by human experts but with minimum manual effort. In this research, we propose a novel approach for peak detection using machine learning technology, which is utilized in current applications of artificial intelligence (AI). This research also compares manual peak detection with the proposed approach and discusses the outlook for AI-based peak detection. We expect that this approach will realize improvements in analytical precision and will reduce the analytical time and effort for peak detection, thereby contributing to metabolomics research.

P-38	INTEGRATED SOFTWARE FOR LIPID DATA ANALYSIS IN DIRECT INFUSION ULTRA-HIGH RESOLUTION ACCURATE MASS SPECTROMETRY BASED LIPIDOMICS WORKFLOWS

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CO-AUTHORS: *Yasuto Yokoi, Yukihiro Fukamachi, Reiko Kiyonami, Gavin Reid, Andreas Huhmer*

Infusion MS-based lipidomics workflows using ultra-high resolution accurate mass spectrometry (UHRAMS) coupled with selective derivatization of lipid functional groups provide a convenient solution to address isobaric and isomeric mass lipid overlap. One remaining bottleneck limiting wide-spread application of high-throughput untargeted lipidomics is the lack of integrated software tools. We describe here LipidSearch 5.0 software designed specifically for infusion lipidomics analysis. Extraction of lipids followed by selective derivatization of amino phospholipids and plasmalogen ether-containing lipids was coupled with UHRAMS analysis using a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer. "Sum-composition level" lipid identification was performed by LipidSearch 5.0 software. Key data processing features include mass re-calibration, Gaussian peak fitting and Poisson modelling to identify and correct isotopic overlaps. First the monoisotopic peak is assigned by searching against a user-defined database in SMILES format, enabling individual selection of lipid categories, class/subclass, total number of carbons and double bonds, positive or negative ion adducts and definition of "fixed" and "variable" modifications. Then, isotopic peaks from the identified lipid are removed and the process is repeated for all remaining peaks. In a few seconds 500-1000 "sum-composition level" lipid species are confidently identified from crude lipid extracts. Normalized results are used for relative quantification and statistical comparison between groups. Positive and negative ion results are merged, providing higher confidence for lipid identification. MS/MS spectra are searched for product ions predicted from ions identified during MS data processing. Unique and non-unique product ions are assessed and MS/MS results are corrected for isotopic overlap.

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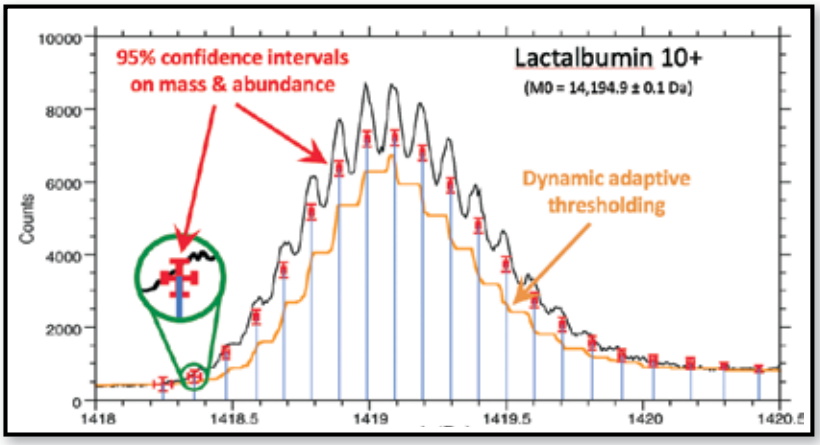

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P-39	Quantitative analysis of short chain fatty acids by chlorformate derivatization using GC-MS

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CO-AUTHORS: *Norihiro Sakui, Sadao Nakamura*

As intestinal bacteria exist in over 30,000 species in humans, they have various influences on human as a host. Recently, interest in research on the relationship between the intestinal bacteria and various diseases (allergic diseases, colon cancer, psychiatric disorders, etc.) has been high, particularly in physiological role of short chain fatty acids (SCFAs) which are produced by the metabolism of intestinal bacteria. In this study, an optimized method based on GC-MS with chloroformate derivatization was developed for SCFAs. The advantage of this method is instantaneous reaction in water and no heating is required. We tested several kinds of alcohols to conduct alkylation of SCFAs using chloroformate derivatization reagent. Because the formed alkyl ester of SCFAs with long carbon chains come to have big molecular weight, elution in GC/MS become late and improvement of the separation is expected.1-butanol showed the suitable reaction for SCFAs among them. Formic acid could not be separated in GC/MS from n-hexane with 1-propanol even though GC condition was changed. As for 1-hexanol, low reaction rate was observed for C1-C4 against high reaction rate for SFCAs with number of carbon more than 5. Finally, derivatization SCFAs were performed with chloroformate after adding mixture of 1-butanol/pyridine under basic condition. Derivatized SCFAs was extracted with n-hexane, then injected into GC-MS. The correlation coefficient of all SCFAs showed over 0.998 at 20-1000pg (6 point) and LODs were below 20 pg on column. We applied this method to measure SCFAs in fecal sample from a mouse and various SFCAs were detected.

P-40	An investigation into MALDI imaging sample preparation compared to DESI imaging for multimodal MSI in Pre-clinical Breast Cancer Research

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CO-AUTHORS: *Michael Batey, Jonathan Sleeman, Kirill Veselkov, Mark Towers, Philippa Hart, Emmanuelle Claude*

Tissue sections were analyzed firstly by MALDI MSI using a SYNAPT G2-Si mass spectrometer with a MALDI source operating with a solid-state diode-pumped ND:YAG laser using a repetition rate of 1 KHz. The MALDI first sample preparation used was with CHCA in MeCH/Water. Consecutive tissues were then analyzed by DESI MSI, using a modified Prosolia source, directly mounted onto the SYNAPT G2-Si. Normal breast tissue is mammary fat pad, with a high presence of triglycerides (TG). DESI control tissue datasets, the highest signals were generated by the triglyceride molecules directly from the tissue sections i.e. m/z 879.74 (TG(54:6))H+ or (TG(52:3))Na+ and 853.73 (TG(52:5))H+ or (TG(50:2))Na+. The molecular profiles for breast tumor samples change with an increased intensity for the detection of phosphatidylcholine (PC). This was not observed with MALDI. MALDI triglyceride molecules were observed in both normal or cancerous tissue under the sample preparation conditions used. The observed difference for MALDI between the tissue types were more subtle and related to phospholipids. For example m/z 808.58 (PC(36:2))Na+ was less abundant in the tumor whereas m/z 772.52 (PC(32:0))K+ was more intense in the tumor sample. The lack of TGs in the MALDI datasets, resulted in a second preparation using CHCA in MeOH/Water, clearly showing the presence of TGs in control tissue and tumor bearing tissue. A third MALDI sample preparation using DHB in MeOH/Water will be tested to evaluate the nature of the lipids class ionized and to further examine the complementary nature of MALDI and DESI MSI.

P-41	Reduction of matrix effect in GCMS ion source and method optimizations for cohort study of disease metabolomics

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In large scale cohort studies for disease metabolomics, it is important to maintain metabolite selectivity and sensitivity at a constant level from the beginning to the end of the measurement. All steps that are blood sampling, blood cell separation, extraction, concentration, derivatization, GC-MS analysis, peak identification might involve causes of error and fluctuations. Among them, silylation and following GC-MS analysis were studied for further improvement in the present study. Instable GC-MS analysis affords bad influence on peak identification in the large scale cohort sample analyses. As a result it negatively affects in organization of metabolome matrix. Therefore stability of GC-MS analysis system is particularly required for automated and high-throughput analysis. Preliminary experiment using standard plasma for metabolomics analysis (NIST SRM1950) indicated that the first priority is to remove protein and trigriceride from plasma. After survey of condition, water / acetonitrile extraction was chose as the method to remove matrix that negatively affects. The extracted sample was subjected to GC / MS after concentration, lyophilization, and derivatization. The acetonitrile extract solution of this method can be used simultaneously as a sample for measurement of free fatty acid etc. by LC / MS. The most significant problem on ionization derived from plasma matrix has been drastically reduced through the present study. In addition, sililation process was also improved to yield better reproducibity by using solid state derivatization method.

P-42	Make Big Data Come Alive: Interactive Data Visualization in Metabolomics Research

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CO-AUTHORS: *Jinxi Liu*

Metabolomics research has rapidly evolved in recent years. In this data-intensive field, effective and simple data visualization tools empower researchers to present the big data in a meaningful way that people can quickly understand and use. Compared with traditional static graphics and tables, interactive visualization takes the concept a step further by allowing self-service faceting, probing and drill down. We developed several interactive data visualization applications for metabolomics research using Shiny and Markdown by RStudio coupled with R packages ggvis and plotly. The applications present information including quality control and regression analysis of more than 3000 metabolites in thousands of different models. Results are conveyed both in data tables and statistical graphs. Data tables contain complete information and are downloadable. In statistical graphs, users are allowed to view pointwise values using mouse-over controls, to drill down for detail through zooming, to compare and contrast the models and to display subsets of results by filtering on p-values, treatment groups, model adjustments, metabolites classes or even selecting an individual metabolite. The web-based application employed user interface (UX) design principles to achieve a simple and intuitive interface and can be published to allow public or secure (authenticated) access for sharing. The above features of these Shiny applications enable a self-service, meaningful and flexible way to review and communicate data. Examples of the implementation of these ideas in metabolomics research for a clinical trial, the Diabetes Prevention Program Outcomes Study, and consortium-based analyses, COMETS.

P-43	Development of a combined untargeted and targeted metabolomic approach for the absolute quantification of a large set of central carbohydrate metabolites and their isomers in mice spinal cord in a single analytical run

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CO-AUTHORS: *Fanny Leroux, Benoit Colsch, Delphine Bernard, François Fenaille, Christophe Junot, Sandrine Aros*

Liquid Chromatography coupled to High Resolution Mass Spectrometry (LC/HRMS)-based metabolomics allows integration of targeted and untargeted approaches for both the quantification of a wide range of metabolites and biomarker discovery. Nevertheless, compounds quantification in biological matrices remains a tremendous challenge in metabolomics because of many coexisting isomers and large dynamic range. Here, we investigated the capability to combine untargeted and targeted metabolomics and the relevance of a novel approach to distinguish and quantify isomers in biological extracts in a single analytical run. This development was performed with a Q-Exactive-Plus instrument coupled to liquid chromatography (Zic-pHILIC–negative ionization mode). For compounds that are separated by chromatography, absolute quantification was performed by using LC/HRMS. For isomeric/isobaric compounds that were co-eluted, quantification was achieved using specific ions present in MS^2 spectra or through differences in relative abundances of common fragment ions. The relevance of this method was evaluated on mice spinal cord extracts. With this technique, the quantification of 40 metabolites involved in central metabolism (Krebs cycle, Glycolysis, Pentoses Phosphate Pathway), including 33 isomers, was achieved. Furthermore, we were able to accurately determine the proportion of co-eluted isomers (absolute error <10%) by estimating differences among theoretical and experimental ratios between diagnostics ions from isomer mixtures. At last, our method enabled reliable quantification of a number of metabolites in biological media over physiological ranges while maintaining a large metabolic coverage capability. In conclusion, it is a powerful tool for metabolomic studies that bridges the gap between traditional targeted metabolite quantification and untargeted metabolomic profiling.

P-44	Evaluation of High Speed, High Resolution Data Independent Acquisition for Combined Identification and Quantitative Metabolomic Flux Analysis

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CO-AUTHORS: *Baljit Ubhi, Emile Plise, Nishit Sitapara, Samira Ashtiani*

LC/MS has become a mainstay in metabolomics research for the identification and quantitation of metabolic species. Recently there has been a shift from measurement of steady-state metabolite levels to measuring the kinetic flux of labeled species in the metabolome. Flux analysis can elucidate the regulation of complex overlapped pathways and see differences that are lost in steady-state measurement. Data independent acquisition (DIA) and targeted techniques show much promise to provide a complete data solution for flux measurements. Herein we evaluate the merits of various high resolution mass spectrometric approaches to the measurement of metabolic flux. MDCK cells were cultured under sterile conditions (standards ATCC recommendations) and seeded onto 6 well plates. Glucose and heavy amino flux were studies were performed using 13C6 labeled materials glucose. Time points were taken at 0,10,20,60,180 and 1440 minutes. Cell samples were analyzed by weak anion exchange chromatography coupled to a QqTOF mass analyzer. Positive and negative mode datasets was generated using a targeted, data dependent acquisition (DDA) and DIA techniques. PCA and PLSDA was effective for identifying species that were undergoing flux. DDA, DIA and targeted MS/MS modes each have distinct advantages for identification and quantitative kinetic measurements. While basic flux measurement can be derived from the HRMS data layer, the location of heavy atom incorporation requires MS/MS data. The use of MS/MS level data on flux measurement is advantageous and in some cases affords the ability to show differences in pathway contribution where ambiguity exists.

P-45	Analysis of Urine SRMs with Comprehensive Two-Dimensional Gas Chromatography (GC×GC)-High Resolution Time-of-Flight Mass Spectrometry

PRESENTING AUTHOR: *Martin Schaut, LECO Australia, Australia*

CO-AUTHORS: *Joe Binkley, David Alonso, Lorne Fell, Jonathan Byer, Liz Humston-Fulmer*

In this study, various sample introduction techniques were used for the identification of volatile and semi-volatile compounds in two new standard reference materials (SRM, smoker's and non-smoker's urine). These reference materials are vital in the diagnosis and setup of standard operating procedures for metabolic profiling of urine in humans. Solid Phase Micro Extraction, Dynamic Headspace and Liquid Injection sample introduction techniques were used to analyze the SRMs. The applied methodology also included a combination of complementary hard and soft ionization techniques coupled to high resolution time of flight mass spectrometry, deconvolution and targeted processing methods which were used for characterization of samples. Analyses resulted in confident identification of a wide variety of materials (e.g., polyaromatic hydrocarbons, phthalate metabolites, phenols and pain killers) and metabolite derivatives. GCxGC-TOFMS chromatograms were highly structured showing clustered classes of compounds and provided high quality spectra that were searched against large, well-established databases. Comparison of the sample introduction techniques yielded expected results; dynamic headspace and SPME having a larger volatile profile. High resolution time-of-flight mass spectrometry (HRT) resulted in additional benefits such as accurate formula determinations for fragment, molecular and adduct ions, as well as, increased selectivity that reduced background interferences. Comprehensive HRT data was probed multiple times via targeted and/or untargeted processing methods to identify important classes of compounds.

P-46	Development of online SPE-GC-MS system with automated SPE-based derivatization method for metabolome analysis

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CO-AUTHORS: *Koji Machitani, Shusuke Osaki, Masahiro Furuno, Eiichiro Fukusaki*

Due to complicated sample preparation procedures that include centrifugal concentration, freeze-drying and derivatization, the current metabolomics protocol using GC-MS, requires not only longer processing time but also advanced technical skill. In addition, some silylated metabolites are unstable and the time required for complete silylation varies depending on the metabolite. Therefore, the time gap between derivatization and actual GC-MS analysis can potentially be a source of error. In this study, automated SPE-based derivatization method coupled with GC-MS was developed to accomplish an extremely rapid sample preparation. The derivatization method using SPE-gel has a high reaction efficiency and can provide sequential sample introduction to keep the interval from silylation to GC-MS injection constant. Amino acids and organic acids from the extracted samples were loaded into the SPE column filled with a mixture of ion-exchange sorbent. After washing with acetonitrile/water, the remaining water in the sorbent was removed with acetonitrile. TMS derivatization was done by permeating MSTFA reagent directly into the sorbent. A needle was automatically connected to the SPE cartridge and inserted into the GC-MS. Then, the TMS-derivatized metabolites were eluted with n-hexane, and injected directly into GC-MS with LVI spiral insert. Pre-treatment, dehydration and derivatization of the sample was done in 10 minutes. Applying the system to metabolite standard solutions and mouse serums resulted in good reproducibility and robustness. Thus, online SPE-GC-MS system with automated SPE-based derivatization method enables the rapid and reliable analysis of metabolites.

P-47	High throughput mass spectrometry-based metabolomics for optimization of strain performance; accelerating the concept-to-production process in industrial synthetic biology

PRESENTING AUTHOR: *Judith Denery, Amyris, United States*

CO-AUTHORS: *Celeste Sandoval, Thomas Scherbart, Marites Ayson, Isabel Ribeiro, Carol Tran, Derek Abbott, Alex Apffel, Anna Hjelmeland*

Strain optimization for microbial production of chemicals has historically centered around screening and selection for improved strains based on some combination of cell growth and product titer. Unfortunately, reliance on these extracellular parameters, provides an incomplete picture of how these products are being made or how production can be improved. Metabolomics and proteomics measurements, however, provide the ability to "look under the hood" of each living cell factory to inform rapid strain optimization through data-driven engineering approaches. Despite the obvious value of metabolomics and proteomics analysis, the impact of such approaches is currently limited by the low throughput, manual and time intensive nature of the work. With funding from DARPA, the Amyris HTP-MS metabolomics platform has been developed for the analysis of >600 samples a day, thus providing a snapshot of metabolic machinery that covers a wide range of microbial metabolism, representing central carbon metabolism, product pathways, intermediates, byproducts, and membrane components. The entire platform, including sample preparation, data acquisition, and data analysis, has been streamlined and automated to increase throughput by over 20-fold. These high throughput mass spectrometry-based metabolomics approaches provide essential data for characterizing the genotype to phenotype relationship within the context of the living cell factory and serve as a key driver for iterative strain improvement. A summary of these transformative metabolomics capabilities and their application in helping Amyris deliver on its mission to accelerate microbial production of high value chemicals from milligram-to-kilogram quantities will be presented.

P-48	PeakInvestigator® Maximizes Discovery of Robust Metabolite Features in Complex Sample Analysis with Dynamic Thresholding & Precision Centroiding

PRESENTING AUTHOR: *Jeff Peterson, Veritomyx Inc., United States*

CO-AUTHORS: *Jeff Peterson, Luke Schneider, Adam Tenderholt*

In this study we compared two different centroiding methods in the analysis of three lipidomic samples (food plate homogenates from different diets) processed by LC/MS. Each sample comprised of six replicate LC/MS runs generated on an Agilent 6550 at 40K resolution. PeakInvestigator® (PI) is a new advanced signal processing method for centroiding mass spectrometric data, which is fully automated. The PI results were compared to standard centroiding methods (MZmine Exact Mass). Metabolomic analyses (MetaboAnalyst) were performed independently on the centroided masslists generated by the different methods to identify statistically-significant sample-discriminating features. Every significant (p-value < 0.01) chromatographic feature found in all six technical replicates and unique to each sample was further examined manually. After discarding common features found in both PI and SC analysis, PI consistently reported higher numbers of significant sample-discriminating features. The additional features discovered with PI were due to a combination of locally-adaptive dynamic thresholding, statistical signal to noise discrimination, and centroiding precision with statistical confidence intervals for each peak, allowing more sensitive, precise and complete construction of chromatograms. We conclude that PeakInvestigator® can significantly improve metabolomic analyses and make them more reproducible since there are no user adjusted parameters.

P-49	A study of chemical exposure metabolites in human urine samples by novel High Performance benchtop time of flight GC/MS system.

PRESENTING AUTHOR: *Ben Macleod, LECO Australia, Australia*

CO-AUTHORS: *David Alonso, Joe Binkley, Lorne Fell*

Effective profiling methods are critical for the investigation of environmental exposure. Metabolomics is particularly useful due to phenotype proximity and quick insight into system perturbations. A major problem continues to be incomplete sample characterization due to compound chemical diversity, wide concentration range of metabolites and the complexity of biological matrices. Samples were dried, treated with urease and derivatized. A novel benchtop GC-TOFMS and associated software were used for comprehensive data collection and processing. Compounds were characterized using retention index values and similarity searches against large, well-established databases. Different group samples were quickly compared retrospectively with Target Analyte Finding processing. A main objective of this research investigation was the development and implementation of an effective workflow for comparison of urine samples. A major bottleneck, regardless of the applied technique, is quick and confident characterization of individual metabolites in samples. In this study, the combination of robust chromatography and the increased dynamic range and lower detection limits of the GC-TOFMS system resulted in rapid identification of xenobiotics (e.g., halogenated compounds, phenols) and metabolites including: Acids, diacids, amino acids, fatty acids, bases, monosaccharides, disaccharides, sugar phosphates, and sterols. Software tools were used to quickly interrogate the rich data retrospectively in a targeted manner after compounds of interest were identified.

P-50	Metabolomic and Metagenomic basis of suppressive soils against Pythium irregulare on lettuce "baby leaf"

PRESENTING AUTHOR: *Margarita Ros, CSIC-CEBAS, Spain*

CO-AUTHORS: *Juan Antonio Fernandez, Trent Northen, Angel Faz, Jose Antonio Pascual, Suzanne Kosina, Benjamin Bowen*

According to sustainable agriculture, suppressive soils have emerged as an alternative to reduce the use of chemical pesticides. The biological basis of suppressiveness has been depicted for majority of the soils, although, can be also associated with the level of organic matter and nature of soil. The objective of this work was to gain insight and find difference between the microbial profiling present in different suppressive and conductive soils (agricultural and natural), with different organic matter contents against Pythium irregulare on lettuce "Baby leaf". Microbial profile was studied with two approaches, high throughput sequencing of bacterial and fungal communities and metabolomics that has emerged as a functional microbial approach that provides insights into the metabolic activity of microbial communities in this case related with biocontrol against host plant pathogen.

P-51	A biocatalytic approach towards the lab scale production of analytical standard metabolites

PRESENTING AUTHOR: *Bernhard Schoenenberger, Sigma-Aldrich Production GmbH, Switzerland*

CO-AUTHORS: *Henrike Brundiek, Roland Wohlgemuth, Markus Obkircher, Roland Meier, Bettina Siebers, Agata Wszolek, Lu Shen, Christopher Bräsen*

A biocatalytic approach towards the lab scale production of analytical standard metabolites The demand for new endogenous metabolites with a well defined quality as analytical standards in Metabolomics is still high. The accessability to many of them either by isolation from natural sources or by traditional synthesis is challenging or nearly impossible due to the complexity of their chemical structure or due to their inherent instability. A series of hitherto difficult to obtain metabolites have been synthesised in the gram scale by mimicking one or more steps of the metabolic pathways and using appropriately cloned and overexpressed recombinant enzymes. Three examples of successful application of this approach will be presented.

P-52	Sugar Rush: New LC-MS method to quantify the plant regulator Trehalose-6-Phosphate

PRESENTING AUTHOR: *CARLA ANTONIO, Instituto de Tecnologia Quimica e Biologica Antonio Xavier (ITQB NOVA), Portugal*

CO-AUTHORS:

Trehalose-6-phosphate (T6P), the intermediate of trehalose biosynthesis, is an important signal metabolite in plants. It is a signal of sucrose status in plants and influences many metabolic and developmental processes, including responses to stress conditions. However, the almost undetectable levels of T6P, together with the complex plant matrix and the presence of T6P isomers such as sucrose-6-phosphate (S6P), makes the detection of this metabolite challenging. This work describes the development and validation of a new hydrophilic interaction chromatography (HILIC) method coupled to negative ion electrospray (ESI) triple quadrupole tandem mass spectrometry (QqQ-MS/MS), in the highly sensitive multiple reaction monitoring (MRM) mode, to quantify metabolic intermediates of trehalose biosynthesis, including glucose-6-phosphate (G6P), uridine 5-diphospho-glucose (UDPG), T6P (and its isomer S6P). The use of piperidine and methylphosphonic acid in the HILIC mobile phase significantly helped to minimize the interaction between the phosphate group of the analytes and the metallic surfaces of the LC-MS system, and resulted in good peak shape and resolution for all target sugar phosphates. The method showed good linearity, repeatability, precision and accuracy, without the need for pre-analytical derivatization or any type of sample clean up, other than metabolite extraction, and was applied to quantify, in the picomole range, the fluctuations of S6P, T6P and G6P in Medicago truncatula roots and leaves exposed to water deficit and subsequent water recovery. This is, to our knowledge, the first time a HILIC-ESI-QqQ-MS/MS method is reported for the quantification of low-abundant T6P in the complex plant matrix of M. truncatula.

P-53	A Novel Simplified, Integrated Solution for Untargeted Metabolomics

PRESENTING AUTHOR: *Katie Glenn, SCIEX, Australia*

CO-AUTHORS: *Tim Garrett, Ranjan Perera, Baljit Ubhi*

Metabolomics focuses on the chemical processes central to cellular metabolism. Mass spectrometry is the tool of choice for the measurement of these metabolites. However, they can be increasingly challenging workflows to setup. Therefore, a robust solution for screening metabolites is of increased interest allowing for a more integrated and routine mass spectrometer system. A new QTOF System was developed for routine, robust workflows which require minimal MS expertise. The system integrates all data acquisition, processing and review as well as reporting into a single piece of software. A prostate cancer study was used to determine whether the untargeted metabolomics workflow using the X500R System could find key differences between the samples. In this study, samples from a pilot prostate cancer study was analyzed and a clear difference between healthy and disease urine samples were detected using this untargeted metabolomics approach, confirming the original disease classifications. The data was collected using information dependent acquisition (IDA) on the X500R QTOF System and processed in MarkerView™ Software 1.3 for statistical analysis. Cancer research represents a key area where metabolomics can provide new biomarkers of disease from easy to obtain biofluids such as urine or plasma. An untargeted screening approach using mass spectrometry that can be adopted by a broader range of research labs (not only the expert labs) would enable this valuable research to be more broadly performed. Most changes were in the small molecule amino acids.

P-55	Differential analysis of LC-IMS-MS data in the analysis of complex wine samples

PRESENTING AUTHOR: *Sven Meyer, Bruker Daltonik GmbH, Germany*

CO-AUTHORS: *Peter Sander, Klaus Meyer*

Several samples of red wine were prepared by spinning down the solid matter. The supernatant was diluted, divided into aliquots and spiked with single standards and mixtures of isomeric standards. All samples were investigated with LC-IMS-MS analyses. The differences were calculated using two different methods. The performance of the two subtraction algorithms are first demonstrated on samples spiked with different isomeric compounds. Subtracting a blank wine analysis from a spiked wine analysis yielded in a differential result data file. All dimensions like retention time, mass to charge, mobility and intensity are considered and retained. The results of the different approaches were compared. Also, the general workflow of unknown ID is demonstrated on these examples, using exact mass, isotopic pattern quality (mSigma value), fragmentation pattern of MS/MS spectra and collisional cross section (CCS). As an example, the investigation of catechin and epicatechin will be shown. In a second part, a differential analysis of non-spiked wine samples was performed. The results show several peaks that were identified as differentiators between the different types of red wine.

P-56	Targeted Profiling – Adaption of a Standardized and Quantitative Metabolomics Assay from Triple Quadrupole MS to Orbitrap MS

PRESENTING AUTHOR: *Therese Koal, BIOCRATES Life Sciences AG, Austria*

CO-AUTHORS: *Doreen Kirchberg, Martin Buratti, Simon Schafferer, Kristaps Klavins, Glenn Damkroeger, Madalina Oppermann, Anastasia Kalli, Reiko Kiyonami, David Peake, Andreas Huhmer, Therese Koal*

Standardized, quantitative assays have been increasingly sought-after in metabolomics, not only in targeted but also in profiling studies, where inter-lab and longitudinal comparability in results are of utmost importance. The AbsoluteIDQ® p180 Kit, a proven tool for multiplexed standardized analysis in Targeted Metabolomics has been adapted for the high resolution Orbitrap mass spectrometry platform, which has been the workhorse of Profiling Metabolomics. Here we demonstrate the power of quantitative analysis of the kit including lipids for Targeted Profiling.on the Q ExactiveTM Focus instrumentThe AbsoluteIDQ® p180 Kit workflow contains two parts: LC-MS and FIA-MS analysis. The LC-MS method provides quantitative analysis of 42 targeted amino acids and biogenic amines. The measured concentrations on the Q ExactiveTM Focus are absolutely comparable to those achieved on the standard triple quad MS-based analyses. Their performance in terms of LOD, LOQ, accuracy and precision are very similar. For the FIA-MS analyses, approximately 360 metabolite features, covering acylcarnitines (AC), phosphatidylcholines (PC), lyso-phosphatidylcholines (LPC), sphingomyelins (SM), ceramides (Cer), cholesteryl esters (CE), glycerides (DG, TG) and hexoses, can be profiled and relatively quantified with the Q Exactive. Although the sensitivity and precision for FIA-MS analysis on the triple quadrupole MS platforms are slightly better, the Orbitrap technology delivers the advantage in discerning isobaric interferences. A betatest of the newly adapted standardized assay has been carried out in three laboratories on different Q ExactiveTM family platforms. The results show a high lab-to-lab comparability, which is mandatory for robust, routine applications of the Kits in metabolomics targeted profiling.

P-57	Optimization of SONAR Elevated Energy Ramps Applied to Different Molecule Classes

PRESENTING AUTHOR: *David Heywood, Waters, United Kingdom*

CO-AUTHORS: *Chris Hughes, Lee Gethings, Jonathan Williams, Johannes Vissers, James Langridge*

Initial experiments were carried out to determine optimum collision energy ramps using one quadrupole mass range, for example m/z 400 – 900 for analysis of complex peptide mixtures and m/z 500 – 1200 for small molecule applications. These were carried out in a systematic manner, i.e. by altering the starting and ending collision energy values in 5 V steps. Optimum ramps, based upon peptide identification rates and feature identifications, showed that ramps of 14 to 40V for proteomic and 20 to 50 V (+ve) and 25 to 55 V (-ve) for small molecules were appropriate values. However, the potential to further optimize collision energy ramps, with the aim of maximizing coverage, based upon precursor m/z and retention time is apparent when analyzing the identified precursors from the injection of 6 µg K562 cell line onto a 300 micron ID column. It is apparent there are distinct retention time regions where SONAR experiments would potentially benefit from using multiple quadrupole m/z ranges and collision energies, i.e. retention order, dependent. The analysis of complex proteomics samples show that the multi-step method has the potential to increase coverage and subsequent quantitation by > 20%. We also applied this same methodology to small molecule (lipidomics) experiments, increasing the feature identification rate significantly and improving the qualitative information by extraction of analyte class information based upon neutral loss or product ion extraction.

P-58	UTILISATION OF SIMLIPID FOR THE CHARACTERISATION OF METABOLIC SYNDROME RELATED LIPIDS ACQUIRED USING A NOVEL SCANNING QUADRUPOLE DIA ACQUISITION METHOD

PRESENTING AUTHOR: *Lee Gethings, Waters, United Kingdom*

CO-AUTHORS: *Ningombam Meitei, Johannes Vissers, David Heywood, Jose Castro-Perez, James Langridge*

Plasma samples were treated with isopropanol and centrifuged for protein precipitation. The lipid containing layer was collected and diluted to adjust the water content prior to analysis. Label-free LC-MS data were acquired in positive and negative ion electrospray mode with an oa-QTof platform using a scanning quadrupole DIA acquisition workflow. Raw data were processed and compound database searched using SimLipid. Subsequent compound identifications were matched to features using the in-built database, which comprised of lipids and in-silico MS/MS characteristic ions. The results were scored based on a proprietary algorithm, which also allowed for isobarics being distinguished. Identifications were curated on the basis of mass accuracy (<5 ppm) for both precursor and product ions. Unsupervised MVA of the resulting data showed clear distinction between cohorts. OPLS-DA was used to filter for features of significant correlation and covariance. Further correction of the data was performed for isotopic overlap prior to targeted quantification, , which indicated differential expression of specific lipid classes including fatty acids, phosphatidylcholines, triglycerides and phosphatidylserines between the three cohorts. SONAR-based analysis indicates that scanning quadrupole DIA enables over an order of magnitude more specificity than a static quadrupole operated with the same resolution and it was found that a quadrupole transmission window of approximately 10 Da provided optimum identifications.

P-59	Black list of metabolomics by in-source decay

PRESENTING AUTHOR: *Yasumune NAKAYAMA, Sojo university, Japan*

CO-AUTHORS: *Eiichiro Fukusaki, Takeshi Bamba*

Non-target metabolomics is promising technology to search biomarkers, novel compounds, and so on. Resent advancement of mass spectrometry (MS) dramatically expand the possibility of the non-target metabolomics. Advancement of mass resolution, MS/MS system, and information technology improved the annotation coverage and precision of identification. However, the some problems are still remains. One of the problems is miss annotation of fragment ions caused by in-source decay. Labile ions are sometimes fragmented at ion source due to the high voltage or temperature to enhance ionization. There fragments become the noise peaks at data integration and hinder peak annotation. Worse, some fragments are homologous to the ions of other metabolites. Meaning, these fragments are miss annotated automatically by software applications regardless the accurate mass and MS/MS. Here we introduce our database "blacklist of metabolomics" to avoid this miss-annotation caused by homologous fragments. The database focus on amino acid, amine, nucleobase, and its derivatives and 104 standard compounds were analyzed one by one using LC/ESI/q-TOF. The result of data dependent acquisition, 704 noise peaks were observed at the same retention time of corresponding compounds and 53 homologous peaks were observed. The blacklist include the in-source fragment ions homologous to the other compound and their precursor. When an annotated compound were in the blacklist, the peak are suspicious. And the peak cannot be trusted when is observed in the same retention time with corresponding precursor ion. Our database and approach help the data of non-target metabolomics more credential.

P-60	Tips and tricks for setting up an NMR metabolomics facility

PRESENTING AUTHOR: *Lorna Wilkinson-White, Sydney University, Australia*

CO-AUTHORS: *Katie Powell, Anthony Dona, William Hadden, Samantha Connolly, Luciano Gonzalez, Ann Kwan*

The University of Sydney's School of Life and Environmental Sciences (SOLES) has recently set up an NMR metabolomics facility. We have installed a liquid handling robot and a SampleJet auto-sampler which is mounted on our 600 MHz spectrometer. This SampleJet has a refrigerated case that allows for individual temperature control of the racks. With these facilities, we have the capacity to run up to 500 samples under automation. Thus far we have run cohorts of human serum, plasma and urine, as well as bovine serum. We have run quality assurance/quality control on our cohorts, and the majority of collected spectra show acceptable line width and line shape. PCA analysis of QC samples show tight groupings, indicating consistency across the data collection. We are currently working on several collaborative projects that include looking at metabolic changes in pancreatic cancer patients, changes to livestock metabolome profiles, as well as identifying metabolites that correlate with pregnancy pathologies. We will showcase some preliminary data from these projects, as well as some tips and tricks on the collection of NMR metabolomics data we have learnt while setting up of our facility.

P-61	MStractor: An R Based Workflow for LC and GC-MS Data Mining

PRESENTING AUTHOR: *Luca Nicolotti, Metabolomics Australia, The Australian Wine Research Institute, PO BOX 197, Glen osmond, 5064, SA, Australia*

CO-AUTHORS: *Jeremy Hack, Natoiya Lloyd, Markus Herderich*

Broad availability of hyphenated MS based techniques such as GC-MS and LC-MS has dramatically increased the analytical throughput over the last few decades and allowed the development of advanced analytical approaches such as metabolomics and non-targeted analysis. The concept underpinning non-targeted approaches is to collect and catalogue the entire chemical diversity in a biological sample set so that each sample constituent can be considered as a potential discriminating feature for sample characterization. The enhancement in metabolite detection sensitivity, combined with non-targeted strategies, has substantially increased the complexity of metabolite data sets: file sizes in gigabyte per sample range are common. This in turn has led to an increased demand for powerful, user-adjustable software for automated data filtering and pre-processing. In this presentation, we describe MStractor, a new R script for processing data originating from non-targeted profiling experiments utilizing LC-MS and GC-MS instrumentation. The MStractor workflow performs the following: Feature extraction (m/z-retention-time pair) using XCMS. Retention time alignment of the detected features across the samples composing the analytical set. Recognition and annotation of isotope clusters, fragments and charge states using CAMERA. Molecular feature filtering based on multiple criteria and conservative usage of intensity thresholds for maximum sensitivity. Creation of a data matrix summarizing the data processing results. Moreover, MStractor shows some unique features such as: 1. Parameterization based on user provided inputs obtained from instrument specifications and reference measurements. 2. Graphical tools for real-time quality monitoring and optimization of the feature extraction process.

P-62	Separation of Protein and Peptides Using Styrene/N-phenylacrylamide porous layer Immobilized Open Tubular Capillary Column

PRESENTING AUTHOR: *Ashraf Ali, Inha University, Incheon South Korea, South Korea*

CO-AUTHORS:

A copolymer immobilized OT-CEC column has been developed in current study for the separation of different peptides present in a tryptic digest of cytochrome C with enhanced separation efficiency and improved peak capacity which is better than those of the previous studies. Long copolymer chains were immobilized on the inner surface of the capillary via reversible addition-fragmentation chain transfer (RAFT) polymerization. Pretreated silica capillary column (50 µm internal diameter and 120 cm length) was chemically modified with 4-(Chloromethyl)phenyl isocayanate in the presence of dibutyl tin dichloride as catalyst. The terminal halogen (Cl) of the bound ligand (4-(Chloromethyl)phenyl isocayanate) was reacted with sodium diethyl dithiocarbamate to incorporate the initiator moieties. A thin polymer layer was made on the inner surface of capillary by reversible addition-fragmentation transfer polymerization upon the initiator moieties using a mixture of styrene, N-phenylacrylamide, and methacrylic acid. The copolymer immobilized open tubular capillary column was used for the separation of synthetic mixture of five peptides and tryptic digest of cytochrome C sample in capillary electrochromatography. Very high separation efficiency (Ca. 1440,000 plates/m) was obtained for synthetic peptides while (Ca. 320,000 plates/m) for some of the peptide in tryptic digest of cytochrome C under optimized elution conditions.

P-63	Automated on-line and on-time MCF Derivatisation for GCMS in a Routine Metabolomic Laboratory

PRESENTING AUTHOR: *Erica Zarate, University of Auckland, New Zealand*

CO-AUTHORS: *Saras Green, Udo Rupprecht*

Automated on-line and on-time MCF Derivatisation for GCMS in a Routine Metabolomic Laboratory Erica Zarate, Mass Spectrometry Centre, School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand (e.zarate@auckland.ac.nz) Saras Green, Mass Spectrometry Centre, School of Biological Sciences, University of Auckland, Auckland 1010, New Zealand (e.zarate@auckland.ac.nz) Udo Rupprecht, Lasersan Australasia Pty Ltd, PO Box 183, ROBINA, QLD 4226, Australia (udo@lasersan.com) The analysis of low molecular weight compounds by GCMS in Metabolomic Studies has been well documented by various authors, however most derivatise off-line prior to GCMS analysis. In the case of MCF (Methyl Chloroformate) derivatisation it is very time consuming, taking around 4 hours per batch of 24 samples to be done. Robotic autosamplers such as from CTC-PAL are available that can perform derivatisation on-line/on-time followed immediately by GCMS injection, increasing reproducibility. However, these have often not allowed the overlap of the lengthy derivatisation procedure with the equally lengthy GCMS run time. This presentation will describe evaluation of a commercial software available from Gerstel GmbH, that automatically overlaps the derivatisation procedure and comparisons in recoveries using a dual 100ul and 10ul syringed CTC-PAL are described in a routine Metabolomic analysis laboratory. This software is available to work with most CTC-PAL autosamplers independent of supplier.

P-284	Acoustic Mist Ionization for Ultra High Throughput Mass Spectrometry

PRESENTING AUTHOR: *Phillip DeLand, Labcyte, United States*

CO-AUTHORS: *Rich Ellson, Martin Bachman, Ian Sinclair, Jon Wingfield, Gareth Jones, Lucien Ghislain, Eric Hall, Sammy Datwani*

Due its ability to generate high quality, label-free data for positive compound identification and quantification, LC-MS has become an essential tool for metabolomic studies. However, as the field of metabolomics has experienced tremendous growth, so to has the need for analyzing larger numbers of samples. Unfortunately, LC-MS throughput has not significantly increased beyond about 1 sample per minute. Other techniques such as automated solid-phase extraction have shown promise but still only offer analysis rates of slightly better than 6 samples per minute. For over a decade, acoustic energy has been used to reliably transfer liquid in small, precise increments from one microtiter plate (the source) to another plate (the destination). Because there is no direct contact with the sample, the possibility of carryover during transfer is non-existent. It is also very fast. Our collaboration with AstraZeneca Pharmaceuticals and Waters Corporation has led to the study of a technology which combines acoustic delivery of liquid with high resolution mass spectrometry. This acoustic mist ionization - mass spectrometry (AMI-MS) combination has produced label-free MS data at a rate of up to 3 samples per second. Although our experience so far has primarily focused on High Throughput Screening, it is clear that this capability could easily be extended to metabolomics. In our presentation, we will describe the fundamentals behind acoustic mist ionization-mass spectrometry, construction of a prototype system and data we have collected with biochemical assays. We will also include examples in metabolic profiling and lipidomics.

P-286	Sample-per-second shotgun lipidomics and metabolomics using acoustically-induced electro-spray ionisation

PRESENTING AUTHOR: *Rich Ellson, Labcyte, United States*

CO-AUTHORS: *Martin Bachman, Ian Sinclair, Jon Wingfield, Daniel Addison, Kerry Hallbrook, Sonia Houghton, Matthew Burnham, Phillip DeLand*

Lipids are abundant components of cell membranes and have vital roles beyond structural support. The identity and abundance of lipid species vary between cell types and cellular states. As such, lipid profiles can be a great readout for cell based assays. Current technologies for lipid profiling (HPLC MS/MS and direct infusion ESI-MS/MS) have relatively low sample throughput. We have developed a workflow which allows rapid analysis of cellular lipid composition and simultaneous detection of intracellular metabolites from small amounts of cells grown in a 384-well plate. We use the recently developed Echo-MS platform (Sinclair et al., JALA, 2016), which combines Labcyte Echo® liquid dispenser and Waters QToF mass spectrometer. An acoustic transducer together with a charging cone generate a spray of charged droplets that de-solvate on transit to the MS source. The contactless nature of introducing samples prevents carryover and allows ultrafast sample analysis. We will demonstrate the ability of the Echo-MS to analyse lipids and metabolites directly from adherent or suspension mammalian cells at sub-second rates. Less than 1000 cells are required to identify and quantify the relative abundance of polar lipid species and a wide range of metabolites in both positive and negative ion modes and with minimal sample preparation required. We will show data of treated cells and how simultaneous analysis of lipids and metabolites can help dissect the effect of a compound on biochemical pathways. We will also show how in situ lipid extraction can be performed to improve ionisation of phospholipids and minimise ion suppression.

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Innovation with Integrity

O-43	An automated, quantum-chemical in silico metabolite library engine for multidimensional NMR spectroscopy

PRESENTING AUTHOR: *Jamie Dunn, Pacific Northwest National Laboratory, United States*

CO-AUTHORS: *Yasemin Yesiltepe, Dennis Thomas, Niranjan Govind, Mark Borkum, Nancy Washton, John Cort, Thomas Metz, Ryan Renslow*

Quantum chemical calculations are currently the most accurate method available for predicting NMR chemical shifts for a wide range of metabolites. Because current metabolite identification methods in complex mixtures rely on chemical shift libraries, high-throughput quantum chemical calculations are needed to enable identification of the estimated hundreds of thousands of metabolites. Furthermore, consideration of conformers is required to obtain accurate chemical shifts, and accounting for hundreds of conformers for each metabolite is time consuming. We built a user-friendly workflow for automated NMR chemical shift calculation that uses an open source, high-performance computational quantum chemistry software, NWChem. Our tool employs density functional theory (DFT) techniques to calculate NMR chemical shifts of molecule sets, with custom options for different solvents, nuclei, and user-selected chemical shift reference compounds. IUPAC InChI identifiers or chemical table files are accepted as input. This tool can calculate NMR chemical shifts of a molecule set with a variety of DFT methods while considering hundreds of conformers for each molecule. Our tool was validated with 300 molecules that had experimental NMR chemical shifts available in the literature. 1H and 13C chemical shifts were calculated with eight levels of DFT theory, with RMSD errors reaching 0.8 and 5 ppm, respectively. Furthermore, we tested our tool on conformers obtained using DFT-based ab initio molecular dynamics, demonstrating the ability to reduce chemical shift errors to less than 0.1 ppm (1H) and 2 ppm (13C) using Boltzmann weighting of calculations for hundreds of conformers.

O-57	Metabolite Profiling of Red Sea Corals

PRESENTING AUTHOR: *Alejandra Ortega, KAUST, Saudi Arabia*

CO-AUTHORS: *Christian Voolstra*

Looking at the metabolite profile of an organism provides insights into the metabolomic state of a cell and hence also into pathways employed. Little is known about the metabolites produced by corals and their algal symbionts. In particular, corals from the Red Sea are understudied, but interesting study objects, as they live in one of the warmest and most saline environments. In this study, we applied gas chromatography – mass spectrometry metabolite profiling to analyze the metabolic profile of four coral species and their associated symbionts: Fungia granulosa, Acropora hemprichii, Porites lutea, and Pocillopora verrucosa. We identified and quantified 102 compounds among primary and secondary metabolites across all samples. F. granulosa and its symbiont showed a total of 59 metabolites which were similar to the 51 displayed by P. verrucosa. P. lutea and A. hemprichii both harbored 40 compounds in conjunction with their respective isolated algae. Comparing across species, 28 metabolites were exclusively present in algae, while 38 were exclusive to corals. Taken together, this study provides a first description of metabolites of Red Sea corals and their associated symbionts. As expected, the metabolites of coral hosts differ from their algal symbionts, but each host and algal species harbor a unique set of metabolites. This corroborates that host-symbiont species pairs display a fine-tuned complementary metabolism that provide insights into the specific nature of the symbiosis. Our analysis also revealed aquatic pollutants, which suggests that metabolite profiling might be used for monitoring pollution levels and assessing environmental impact.

O-62	Differences in the biological architecture between visceral and subcutaneous adipose tissue revealed by transcriptomics and metabolomics

PRESENTING AUTHOR: *Nasim Bararpour, University Center of Legal Medicine, Switzerland*

CO-AUTHORS: *Tiziana Caputo, Federica Gilardi, Nicolas Guex, Beatrice Desvergne, Aurelien Thomas*

Adipose tissue (AT) is a metabolically dynamic tissue which acts as an endocrine organ notably by regulating metabolic homeostasis. The response to overnutrition of distinct AT depots, such as visceral AT (v-AT) and subcutaneous AT (sc-AT), is different, with the production of pro-inflammatory mediators, triggering chronic low-grade inflammation, mainly in v-AT. However, less is known about the molecular signature and gene expression induced-phenotype that differ between these two AT depots.The aim of this study is to decipher the differences in the biological architecture and functions between sc- and v-AT.Comparison was performed between v-AT and sc-AT in mice fed either by high fat or chow diet .Analysis was done by untargeted metabolomics and transcriptomics. Univariate t-test and PAM statistics were performed to identify discriminate markers. Gene Ontology analysis (GO) and pathway enrichment inferred from biological network were done using ClueGo, a Cytoscape plug-in.Several markers were significantly up- or down-regulated between sc- and v-ATs, regardless of diet model and inflammatory phenotype of these two tissue types.Mapped genes disclose clear differential expression between v-AT and sc-AT revealed by PCA.Preliminary data analysis addresses some purine and pyrimidine metabolites that vary across the ATs likewise some steroid metabolites and amino acids.GO analysis enriched several interesting pathways notified by contribution of two "omics" data such as mesenchymal development pathway which may address ontogenetic difference between v-AT and sc-AT tissues.Integrating information driven by metabolomics and transcriptomics analysis assists in revealing the shared and different biological pathways between sc- and vAT and gives powerful understanding of AT molecular architectures.

O-89	Comparative analysis of phytochemicals in the leaf extracts of Pittosporum angustifolium cultivars via LC-MS based plant metabolomics.

PRESENTING AUTHOR: *ANUJA PATIL, SCHOOL OF PHARMACY, UNIVERSITY OF QUEENSLAND, Australia*

CO-AUTHORS: *MO Parat, PN Shaw, M Fitzgerald*

Introduction- Pittosporum angustifolium L., formerly known as Pittosporum phillyraeoides (Cayzer, Crisp, & Telford, 2000), has been reported for its widespread use in Aboriginal medicinal practice. Such uses of this plant have attracted scientific investigations into its phytochemical profile and medicinal properties (Sadgrove & Jones, 2013). The range of the Aboriginal peoples' usages of this bush medicine is likely extended by the significant geographic and soil variability between states and territories in Australia. Methods- This study included three cultivars and two types of extracts (Juice [GGJ] and decoction [GGD] of Pittosporum angustifolium L. Plant metabolites were extracted and were analysed via reversed phase ultra-high performance liquid chromatography/high-resolution mass spectrometry with electrospray ionisation (ESI) in positive ion mode; this methodology was used to compare the phytochemical profiles of GGJ and GGD. Results- A number (101) of discriminating phytochemical markers was identified using multivariate hierarchical clustering analysis (HCA) (Fig. 1). Among them, three new phytochemical compounds in this plant were identified. Despite GGD and GGJ being derived from the same samples of plant material, the concentrations of these compounds were significantly higher in all GGD samples (p <0.0001). Conclusion- The present study has assessed the phytochemical diversity between leaf extracts and the originating cultivars of Pittosporum angustifolium L., obtained from different locations in Australia, by analysing metabolic fingerprints using UPLC-ToF-MS-based metabolomics and chemometric approaches. Clear differences were observed between the two types of extracts; in addition, intra-cultivar variation was observed.

O-104	Next generation, 'standards-free' metabolite identification pipeline

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CO-AUTHORS: *Dennis Thomas, Jamie Nuñez, Yasemin Yesiltepe, Niranjan Govind, Xueyun Zheng, Erin Baker, Justin Teeguarden, Thomas Metz*

The capability to identify metabolites in complex clinical and environmental samples will revolutionize the search for determinants of disease. In comparison to near-comprehensive genetic information, much less is understood of the metabolome, largely due to insufficiencies in molecular identification methods. Through innovations in computational chemistry, we have developed a platform to overcome a significant obstacle in the field of metabolomics: the absence of methods for accurate, comprehensive identification of small molecules without relying on authentic chemical standards. Our approach uses a gas-phase molecular property, collision cross section (CCS), which can be accurately predicted and consistently measured and, thus, can be used for identification without the need for standards. The pipeline uses a large-scale computational-chemistry platform for calculating metabolite CCS, which exploits PNNL's high-performance computational quantum chemistry software, NWChem, and results in calculated CCS values with errors <1-2% compared to experimentally measured values – sufficient, with accurate mass information, to allow high accuracy identification of metabolites. We applied our platform to analyze positional and geometric metabolite isomers and found it was significantly more accurate at calculating CCS values compared to other methods. This level of accuracy enabled us to distinguish cis/trans isomers. Furthermore, we tested its high-throughput capability with metabolites in the HMDB, which provided a measure of the theoretical resolving power of accurate mass and CCS. Finally, we analyzed environmental samples and CCSs were calculated in silico for possible metabolites. Several predicted degradation products, not available as authentic chemical standards, were identified only by accurate mass and in silico-derived CCS.

O-106	Lipidome Remodeling at Sea: Viral Infection of a bloom forming Marine Algae Induces the Production of Highly Saturated Triacylglycerol

PRESENTING AUTHOR: *Sergey Malitsky, Weizmann Institute of Science, Israel*

CO-AUTHORS: *Carmit Ziv, Shilo Rosenwasser, Shuning Zheng, Daniella Schatz, Ziv Porat, Shifra Ben-Dor, Aharoni Aharoni, Assaf Vardi*

Lipidomics is a subset of metabolomics which refers to the large-scale study of pathways and networks of cellular lipids in biological systems. We use reversed-phase chromatography to separate complex lipid molecular species which are then analyzed by high resolution mass spectrometry provided by qTOF. As a biological model to study global lipidome changes we used the marine microalga Emiliania huxleyi. This ubiquitous alga forms massive seasonal blooms in the North Atlantic Ocean that impact nutrient recycling and earth climate. Viral infection of this unicellular alga induces the rapid remodeling of host primary metabolism, targeted towards fatty acid metabolism. We applied an untargeted LC/MS-based lipidomics approach to explore the impact of viral-induced metabolic reprogramming on lipid composition during interaction in this unique host-pathogen system. This included the analysis of few hundreds lipids from 5 different lipid classes during the course of viral infection. We show that lytic viral infection leads to massive remodeling of the cellular lipidome, predominantly inducing the biosynthesis of viral specific glycosphingolipids as well as the accumulation of highly saturated triacylglycerols (TAGs) in lipid droplets. These virus-induced lipids accumulated in the infected algal cell as well as in the purified virion, thus pointing to the central role of these lipids for viral assembly. Interestingly, viral-induced TAGs were significantly more saturated than TAGs produced under nitrogen starvation. This study highlights TAGs as major products of the viral-induced metabolic reprogramming during host-virus interaction and suggests a possible biotechnological application for these TAGs in biofuel production.

O-143	Identifying Marine Natural Products - Pitfalls and Progress

PRESENTING AUTHOR: *Mary Garson, The University of Queensland, Australia*

CO-AUTHORS:

Chemists routinely use NMR spectroscopy to determine the structures of organic compounds. Natural product researchers, armed with a suite of 2D NMR methods and knowledge of biosynthetic pathways, can usually deduce the planar structure of a complex natural product without difficulty. The more complex task of assigning the molecular shape (that is relative and absolute configuration) presents more of a challenge, yet no identification of a natural product is complete without this stereochemical information. In recent years, the use of quantum chemical calculations for the prediction of proton and carbon chemical shifts provides an additional tool that aids complex structure elucidation. In this talk, I will describe some recent misidentifications in the natural products literature, and highlight the role of advanced NMR spectroscopy in the structural revision.

O-156	A multi-platform metabolomics approach to identify unique compounds present in Australian bush food

PRESENTING AUTHOR: *Fe Calingacion, University of Queensland, Australia*

CO-AUTHORS: *Melissa Fitzgerald*

Australia is home for immense collection of native plants offering edible species used as food and medicine. Aboriginal people have developed vast knowledge of use for this diverse flora, yet majority of these species have not yet been extensively examined. In this study, we have exploited several bush food such as myrtle, gumbi gumbi, senna acacia and native plums to understand their rich chemical diversity. We determined polar and semi polar metabolites that are unique for each bush food class using ultra high pressure liquid chromatography-mass spectrometry. For highly similar molecules, we have used ion mobility which offered additional dimension of separation based on the collision cross section property of the compound. Furthermore, we identified and compared the volatile components of lemon myrtle with other commercially available herbs using a two-dimensional gas chromatography-time of flight-mass spectrometry. Lemon myrtle was found to have more of the unsaturated aldehydes and furanones that are characteristic of desirable flavour and fragrance agents. These information will pave the way for a wider application of these native food and ultimately increasing their economic value.

O-157	HSQC-TOCSY NMR profiling: making informed microbial strain selection for natural product discovery

PRESENTING AUTHOR: *Larissa Buedenbender, Environmental Futures Research Institute, School of Environment, Griffith UniversityGriffith University, Australia*

CO-AUTHORS: *Leesa Habener, Tanja Grkovic, Ipek Kurtböke, Sandra Duffy, Vicky Avery, Anthony Carroll*

Microbial natural products are an excellent source for drug leads due to their unique structural diversity and the use of laboratory-based fermentations rather than harvesting organisms from the environment. However, re-discovery of already known natural products poses a great challenge; therefore, it is important to incorporate effective dereplication protocols early in natural product isolation efforts. We have developed a systematic approach for microbial strain prioritization for natural product discovery based on HSQC-TOCSY nuclear magnetic resonance profiles combined with biological activity of crude extracts. NMR data provides structural information of the microbial extracts and can be used to target specific functional groups of natural products. The use of HSQC-TOCSY experiments offered increased chemical shift resolution by spreading the structural information onto two dimensions and thus permitted assessment of unfractionated extracts. Here, we present how this approach was used to make informed selection of microbial strains with unique NMR profiles and with demonstrated anti-plasmodial activity from a library of 120 ascidian-associated actinomycete extracts that resulted in the isolation of new natural products.

O-190	Comprehensive LC-MS/MS metabolomic profiling of fungal culture collections – insights into the chemical space of fungal secondary metabolites

PRESENTING AUTHOR: *Mark Sumarah, Agriculture and Agri-Food Canada, Canada*

CO-AUTHORS: *Justin Renaud, Keith Seifert, J. Miller*

Filamentous fungi are capable of producing an immense variety of secondary metabolites (polyketides, terpenes, nonribosomal peptides) including many important mycotoxins. Over the past century, Agriculture and Agri-Food Canada has built a large resource of 20 000+ fungal cultures (Canadian Collection of Fungal Cultures). These include Fusarium, Penicillium, Alternaria, Aspergillus, and many endophyte sourced species. We have developed a comprehensive non-targeted LC-MS/MS data independent acquisition (DIA) method using our High-Resolution Orbitrap Q-Exactive mass spectrometer and a data processing pipeline for metabolomic profiling. Extracts are screened against an in-house digital MassBank Spectral Library (dMBSL) database comprised of LC-MS and MS/MS spectra of mycotoxins and fungal secondary metabolites for rapid and confident metabolite identification. Spectra of all ionisable compounds are digitally archived, allowing for comparative multivariate statistical analysis (PCA and OPLS-DA) and retrospective data mining. Important spectral features can be easily searched using retention times, high resolution mass, and by product ion fragments. Each fungal extract was also screened against panels of bacterial and fungal microorganisms as part of our ongoing search for novel bioactive compounds. The goal of this project is to better understand the fungi that occur in Canada and more importantly the secondary metabolites that they produce and their potential roles in agriculture. This data is shared with regulators for risk assessments and development of science based policy. To date more than 1000 strains have been screened and the secondary metabolites of fungal endophytes and new described Penicillium and Aspergillus species have been characterized and published.

O-228	Metabolomics: The solution to assessing herbal medicine safety?

PRESENTING AUTHOR: *Garth Maker, Murdoch University, Australia*

CO-AUTHORS: *Elly Crighton, Ian Musgrave, Michael Bunce, Roger Byard, Robert Trengove*

Herbal medicines are part of the $2 billion Australian complementary and alternative medicine industry, lightly regulated due to a perception of 'low risk'. This has been challenged by the authors, who investigated the safety of traditional Chinese medicines (TCM), with 92% of products tested having toxic ingredients, pharmaceutical agents or heavy metals. There is a clear need for new approaches to assessing safety of herbal medicines, and metabolomics could provide the solution. A fully untargeted approach (DSA-TOF-MS) was used to screen 150 herbal medicines, identifying numerous undeclared ingredients and potentially toxic metabolites, including caffeine. Secondly, to examine variation in composition between formulations, 6 commercial ginseng products were subjected to targeted (LC-QQQ-MS) and untargeted (GC-QTOF-MS) analysis. Numerous differences were detected in ginsenoside composition and metabolite profiles. Several herbal medicines have been linked to liver damage, although mechanisms of toxic action are often not known. Human liver cells (HepG2) were exposed to a green tea extract for 20 min at 10 mg/ml. Cells were harvested and analysed using GC-QTOF-MS. Data was modelled and mapped to biochemical pathways. This revealed numerous changes in central carbon metabolism, suggesting a disruption to glycolysis that led to increased TCA cycle utilisation of amino acids and fatty acids. Metabolomic analysis clearly has the potential to make a major contribution to studies of herbal medicines, both in terms of safety and assessing potential health risks. For improved compliance and safety within the herbal medicine industry, the use of novel methods needs to be explored.

O-258	CANOPUS - Comprehensive categorization of unknowns using tandem mass spectrometry

PRESENTING AUTHOR: *Kai Dührkop, Friedrich-Schiller University, Jena, Germany*

CO-AUTHORS: *Marcus Ludwig, Juho Rousu, Sebastian Böcker*

Novel computational methods such as CSI:FingerID search tandem mass spectra (MS/MS) in structure databases, and assist in identifying compounds in untargeted metabolomic experiments and natural products discovery. However, they cannot help for structures not contained in any database. Here, we present CANOPUS, a tool for predicting compound categories directly from spectral data. This task seems somewhat simpler than structural elucidation, but comes with certain pitfalls: Can we classify when our MS/MS training data is far from a uniform sample of this category, or insufficient or no training data is available? And can we classify a compound of a true unknown that is not contained in any structure database? CANOPUS deals with these pitfalls using a two step approach: First, predict a molecular fingerprint from MS/MS using a kernel support vector machine; second, predict the compound categories from these fingerprints using a deep neural network (DNN) trained on millions of training examples. CANOPUS can train compound categories for which we do not have MS/MS data and it generalizes well for compounds which structures are unknown to the DNN. It predicts 1,143 categories from the Classyfire ChemOnt ontology, such as flavonoids, phenylpropanoids, or glucocorticoids. On independent MS/MS data predictions have an average accuracy of 99.2 %, half of the categories have an F1 score (harmonic mean of precision and recall) greater than 75 %. We applied CANOPUS to several sample datasets from GNPS such as a marine platonic diatom sample, and automatically assign compound categories to each MS/MS spectrum.

O-263	Deep metabolome annotation and the necessity of a thought experiment

PRESENTING AUTHOR: *Gregory Genta-Jouve, University Paris Descartes, France*

CO-AUTHORS: *Pierre-Marie Allard, Jonathan Bisson, Mehdi Beniddir*

The metabolome of an organism consists in the totality of the small molecules present in an organism at a given time. Unlike genome, transcriptome or proteome, it represents the real time content of the a living organism at the metabolic level. In order to understand the most complex biological interactions between cells, tissues, organs or individuals in a specific ecosystem, it is fundamental to first identify all the components, i.e. all the molecules that can be encountered. Over the last decade, identification of small biologically generated compounds, the metabolites, has emerged as the main issue in the field of metabolomics and despite all the great efforts of the community during the last years only a relatively small number of compounds have been referenced in accessible databases, and often with only hypothetical structures. Herein we propose to discuss the philosophical aspects of the important question "what is a known natural product?" in order to move forward to a new paradigm in metabolite identification: identifying the real from the possible. This approach based on available tools of in silico metabolisation and MS/MS spectra prediction and data annotation will lead to the characterisation of new natural products with a limited amount of available biological material at a higher discovery rate. These improvements will enable new challenges to arise and be solved in a wide range of biological systems encountered in domains such as chemical ecology, drug discovery and traditional medicine studies.

O-278	Turning mould into gold: An exploration of mould metabolism to commercialize useful secondary metabolites

PRESENTING AUTHOR: *Kyle Van de Bittner, University of Canterbury, New Zealand*

CO-AUTHORS:

Fungi are excellent bartenders. Millions of years of evolution have given them a chance to perfect their chemical recipes for potent chemical cocktails. Examination of the metabolites in fungal chemical cocktails has given rise to some of our most useful antibiotics and insecticides. However, many useful chemicals that fungi produce still remain beyond our reach because the products are too complex to chemically synthesize or the fungus does not naturally produce enough of the metabolite to meet our needs. In this presentation I will discuss how we can use heterologous expression systems in the filamentous fungi Penicillium paxilli to easily elicit production of scarce metabolites from other fungal species. Focus will be turned to a class of secondary metabolites known as indole diterpenes as I unravel how P. paxilli can be used to identify the function of the genes that other fungi rely on to prepare especially unique indole diterpene metabolites. By identifying how these metabolites are biosynthesized we can control the contents and quantity of the fungal cocktails to gain access to commercial quantities of useful indole diterpenes for novel therapeutics.

O-292	Substance class annotation for long candidate lists in metabolite identification using structural ontologies

PRESENTING AUTHOR: *Sarah Scharfenberg, Leibniz Institute of Plant Biochemistry, Germany*

CO-AUTHORS: *Janna Hastings, Pablo Moreno, Stephan Beisken, Christoph Steinbeck, Steffen Neumann*

As an integral part of Systems Biology, Metabolomics aims to detect and identify the chemical compounds that drive and participate in biological processes. In untargeted Metabolomics various sample types with a large number of different metabolites are characterized. The method of choice today is mass spectrometry because of its high sensitivity and the broad coverage of measurable metabolites. Tandem mass spectrometry, which reveals information about the compound structure, provides useful hints for identification. Despite the ongoing development of spectral- and compound databases, the main bottleneck in metabolomic research remains the annotation and identification of metabolites. To identify a new compound, e.g., a biomarker it is necessary to collect a set of annotations that support a hypothesis, such as the molecular mass, identified adducts or a substance class. Based on a tandem MS spectrum, MetFrag reports a list of structurally related candidate compounds that are ranked according to their explanatory power in terms of the measured spectrum. Given such a list, BiNChE performs an overrepresentational analysis on the structural ontology of ChEBI and reports a list of possible substance classes that are overrepresented throughout the candidates with good scores. This approach was successfully applied on two Benchmark datasets of plant metabolites and environmental standard compounds, respectively. The performance was compared for 'known unkown' and 'unkown unkown' compounds. For a published dataset of C. elegans the suggested class annotations for possibly 'unkown unkowns' could be supported.

O-302	Defining the dark metabolism of the malaria parasite

PRESENTING AUTHOR: *Malcolm McConville, University of Melbourne, Australia*

CO-AUTHORS: *Simon Cobbold, Laure Dumont, Leann Tilley*

A majority of peaks in mass spectrometry-based profiling studies of eukaryotic cell extracts remain chemically undefined and/or are not connected to annotated genes or enzyme activities. Determining the extent to which this metabolic dark matter is derived from novel metabolic reactions/pathways represents a major challenge in the field of metabolomics. We have started to systematically annotate the 'dark' metabolome of the parasitic protozoan, Plasmodium falciparum, the major cause of human malaria. The stream-lined metabolism of certain developmental stages of these evolutionarily divergent parasites make them interesting systems for identifying metabolic processes that evolved early in eukaryotic evolution. We have developed a novel pipeline for detecting endogenously synthesized P. falciparum metabolites by labeling asexual blood stages with a panel of ten 13C-labeled carbon sources. We identified 45% of the ~800 parasite metabolites detected by GC/MS and LC/MS which were labeled with one or more 13C-carbon sources. Several novel phosphorylated metabolite species were identified which are predicted to be by-products of key enzymes in central carbon metabolism. Deletion of genes encoding members of the haloacid dehalogenase (HAD) family of phosphatase resulted in the accumulation of some of these metabolites, with dysregulation of central carbon metabolism and reduced parasite growth in red blood cells. These results suggest that enzyme promiscuity may contribute to the formation of novel metabolites and that HAD family phosphatases have evolved metabolite proof-reading functions as well as direct roles in regulating metabolic fluxes through degradation of phosphorylated metabolites.

O-313	Novel UHPLC-MS-SPE-NMR and UHPLC-timsTofMS/MS Tools for Higher-throughput, Confident Metabolite Identifications and to Address the Number One Grand Challenge of Metabolomics

PRESENTING AUTHOR: *Lloyd Sumner, University of Missouri, United States*

CO-AUTHORS: *Feng Qiu, Dennis Fine, Daniel Wherritt, Zhentian Lei, Mark Schroader, Aiko Barsch, Sven Meyer*

Although the vast utility of metabolomics is well documented in the literature, its full scientific promise has not yet been realized due to multiple challenges. The number one, grand challenge of metabolomics is large-scale, confident chemical identification of metabolites. To address this challenge, we have developed sophisticated spectral, computational and empirical metabolomics tools for the systematic and biological directed annotation of multi-species metabolomes. This presentation will describe the creation of a UHPLC-MS mass spectral library, custom software entitled Plant Metabolite Annotation Toolbox (PlantMAT), sophisticated UHPLC-timsTOFMS/MS and UHPLC-MS-SPE-NMR instrumental ensembles that are being used for large-scale confident metabolite identifications. UHPLC-QTofMS/MS metabolite profiling was performed using Medicago truncatula extracts, and data processed by peak deconvolution and formula prediction. Metabolite identifications were first attempted through spectral matching with custom libraries generated with authentic compounds. However, authentic compounds are not available for all metabolites; and a large number of the detected peaks could not be identified using spectral matching. Thus, the orthogonal data was imported into PlantMAT and structures for approximately 100 saponins and polyphenolic glycosides were predicted. These compounds were isolated, purified and concentrated by mass directed UHPLC-MS-SPE (solid-phase extraction). The SPE isolated compounds were eluted and 1D and 2D NMR spectra acquired. UHPLC-timsTofMS/MS analyses were also performed to discover potentially overlapping compounds and to increase our metabolomics depth of coverage for isobaric compounds not readily separated by UHPLC. Examples will be provided for hydroxylated flavonoids. The results demonstrate that the cumulative platforms allows for higher-throughput and high confidence metabolite identifications.

O-367	Development of Software for Efficient NMR Metabolomics Screening of Natural Products

PRESENTING AUTHOR: *Kimberly Colson, Bruker BioSpin, United States*

CO-AUTHORS: *Christian Fischer*

NMR spectroscopy has significant qualities that make it an attractive tool for metabolomics and especially for natural products. These qualities include high reproducibility, compound specificity and quantitation. Traditionally, NMR has required sophisticated operators to operate and harvest valuable information from resulting NMR spectra. Also traditionally, studies of natural products rely heavily of purification strategies to simplify NMR spectra. Our work focuses on the development of NMR software tool for evaluation of natural products metabolomics spectra, on crude extracts, with an aim to automate the spectral evaluation process. Dereplication and identification of key metabolites in crude extracts are accomplished in automation using various automatically defined line fitting algorithms. Limit of detection calculation are utilized to establish minimal reportable quantity of key components. Taxonomic classification is achieved using scaling function routines allowing for consistent or highly variable spectra. This allows customization for the species or material studied. This poster will show the software development through a series of natural product examples. The aim is to develop an automated work flow for analysis of these spectra for operators of various skill levels including (1) metabolomics researchers and (2) quality control technicians. NMR assists the researcher with accurate component identification. NMR analysis of natural health products may lead to improved quality, labelling and product consistency.

O-407	Ultrahigh-resolution metabolomics for heteroatom-containing specialized metabolites

PRESENTING AUTHOR: *Ryo Nakabayashi, RIKEN Center for Sustainable Resource Science, Japan*

CO-AUTHORS: *Ryo Nakabayashi, Kazuki Saito*

Heteroatom-containing specialized metabolites are often profiled with their common structural features (e.g., ultraviolet spectrum or product ions) in integrated metabolomics. However, because of the huge chemical variety of the metabolites, focusing on the common structural in targeted analysis was impractical so far. We developed ultrahigh-resolution metabolomic approaches for nitrogen (N)- and sulfur (S)-containing metabolites, N-omics1 and S-omics2-5, respectively. In the ultrahigh-resolution MS spectrum, the isotopic ions appear as the counterpart of monoisotopic ions. Exploiting differences of exact mass and signal intensity between monoisotopic and isotopic ions enabled us to extract target monoisotopic ions from metabolome data. N- and S-omics using the solariX 7.0 T, which is the Fourier transform ion cyclotron resonance-mass spectrometry (FTICR-MS) instrument, successfully assigned known and unknown metabolites in plants. Matrix assisted laser desorption/ionization-imaging mass spectrometry (MALDI-IMS) is a powerful technique to understand both the site of target metabolites accumulating and the site of expressing biosynthetic genes related to the target metabolites. The accumulation pattern of the assigned metabolites were visualized by MALDI-IMS using the FTICR-MS instrument. Collating the spatial information on metabolites and genes through these approaches and gene expression analysis helps to elucidate biosynthetic mechanisms. Nakabayashi et al., Anal. Chem. (2017) Nakabayashi et al., Anal. Chem. (2013) Nakabayashi et al., J. Nat. Prod. (2015) Nakabayashi et al., J. Nutr. (2016) Nakabayashi & Saito, Curr. Opin. Biotechnol. (2017)

O-520	Known knowns, known unknowns and unknown unknowns. The art of correctly determining the structure of a natural product

PRESENTING AUTHOR: *Anthony Carroll, Griffith University, Australia*

CO-AUTHORS:

A major bottleneck in metabolomics research remains the definitive identification of individual metabolites found in biological matrices. Unfortunately the majority of these metabolites still remain unidentified and even known compounds can be misidentified. Most MS and NMR based metabolomics analyses use libraries of spectroscopic data generated for known compounds to match spectroscopic data derived from complex mixtures to identify individual compounds present in these biological matrices. Ultimately however, the success of spectral data matching is dependent on the true identify of a molecule being determined correctly in the first place. Natural Product dereplication tools are useful to avoid the rediscovery of known compounds with known biological activities (the known knowns). Biodiversity and chemical diversity directly correlate and since only a small proportion of total biodiversity has been studied there is still a vast array of NPs of unknown structure and biological activity. These compounds are the known unknowns. Unfortunately the many published NP structures that have been incorrectly assigned because spectroscopic data has been misinterpreted. These mis-assignments remain undiscovered until a total synthesis or re-interpretation of spectroscopic data reveals that the molecule doesn't match with that of the originally proposed NP structure. These compounds are the unknown unknowns and these structural errors are can have costly implications. NP structure determination strategies have implications for metabolomics research and this presentation will cover techniques that have been developed to detect known NPs, identify unknown NPs and correct the structures of mis-assigned NPs.

P-64	Development of an LC-HRMS metabolomics method with high specificity for metabolite identification using data independent acquisition (DIA)

PRESENTING AUTHOR: *Romanas Chaleckis, Gunma University Initiative for Advanced Research (GIAR), Japan*

CO-AUTHORS: *Shama Naz, Hector Gallart-Ayala, Stacey Reinke, Caroline Mathon, Richard Blankley, Craig Wheelock*

High-resolution mass spectrometry (HRMS)-based metabolomics approaches have made significant advances. However, metabolite identification is still a major challenge and significant bottleneck in translating metabolomics data into biological context. In the current study, a liquid chromatography (LC)–HRMS metabolomics method was developed using a data independent acquisition (DIA) approach. In order to increase the specificity in metabolite annotation, four criteria were considered: i) accurate mass (AM), ii) retention time (RT), iii) MS/MS spectrum, and iv) fragment/precursor ion intensity ratios. We constructed an in-house mass spectral library of 413 metabolites containing AMRT and MS/MS spectra information at four collision energies. The % relative standard deviations between ion ratios of a metabolite in an analytical standard vs. sample were used as an additional metric for establishing metabolite identity. A data processing method for targeted metabolite screening was then created, merging m/z, RT, MS/MS and ion ratio information for each of the 413 metabolites. In the data processing method, the precursor ion and fragment ion were considered as the quantifier and qualifier ion, respectively. We also included a scheme to distinguish co-eluting isobaric compounds by selecting a specific fragment ion as the quantifier ion instead of the precursor ion. An advantage of the current DIA approach is the collection of full-scan data, allowing identification of metabolites not included in the database. Our data acquisition strategy enables a simultaneous mixture of database-dependent targeted and non-targeted metabolomics in combination with improved accuracy in metabolite identification, increasing the quality of the biological information acquired in a metabolomics experiment.

P-65	LAESI-MSI as a tool to differentiate the root metabolome of native and range expanding plant species

PRESENTING AUTHOR: *Purva Kulkarni, Netherlands Institute of Ecology, Netherlands*

CO-AUTHORS: *Rutger Wilschut, Koen Verhoeven, Paolina Garbeva, Wim Putten*

Our understanding of chemical diversity in biological samples has greatly improved through recent advances in mass spectrometry (MS). MS-based-imaging (MSI) techniques have further enhanced this by providing spatial information on the distribution of metabolites and their relative abundance. This technique can be applied to study different biological samples ranging from single cells to intact whole-body tissue sections. However, some ionization approaches employed in MSI are operated under vacuum, need sample preparation and use a matrix (as in MALDI). These can somehow alter the biochemical status of the sample under study. We employed laser assisted electrospray ionization (LAESI), to perform MSI and study the metabolomic diversity and differences in intact root samples of range-expanding Centaurea stoebe and native plant species Centaurea jacea. Here, we tested the hypothesis that successful range expansion is associated with specific plant secondary chemistry and defense compounds. To examine this, LAESI-MSI was performed in positive ion mode and data was acquired in a mass range of m/z 50-1200 with a spatial resolution of 100 ?m. The acquired data was analyzed using in-house scripts and localization patterns were studied for identified discriminatory mass features. The results revealed clear differences in the metabolite profiles for the two species, in the form of distinct metabolic fingerprints. The results are in-line with previous studies performed using gas chromatography (GC)-MS and direct analysis in real time (DART)-MS. The use of ambient conditions and no sample preparation made LAESI-MSI an ideal technique for direct correlation of the acquired data to underlying metabolomic complexity.

P-66	Advantage of High Resolution Accurate Mass Spectrometry for Metabolite Identification in Untargeted Metabolomics Studies

PRESENTING AUTHOR: *Ioanna Ntai, Thermo Fisher Scientific, United States*

CO-AUTHORS: *Ralf Tautenhahn, Tim Stratton, Anastasia Kalli, Amanda Souza, Andreas Huhmer*

High resolution accurate mass (HRAM) spectrometry has become the analytical technique of choice for untargeted metabolomics studies. HRAM allows accurate mass assignments, resolving near mass isobaric species from complex mixtures, thus enabling confident compound identification and quantitation. Here, we aimed to investigate the effect of mass resolving power on metabolite identifications. Human plasma (NIST SRM 1950) was analyzed at different mass spectral resolutions ranging from 15,000 to 240,000 with a Q Exactive HF hybrid quadrupole-Orbitrap mass spectrometer operated separately in both positive and negative mode. The data were analyzed using Compound Discoverer 2.1 for metabolite identification. Increased resolving power was extremely useful in defining isotopic distribution and determining elemental composition. Feature detection improved with increasing resolution, yielding the best results at resolving power of 60,000-120,000. Metabolite identification was performed with Compound Discoverer 2.1 searching against mzCloud and Chemspider. Highly confident metabolite identifications were obtained with mzCloud, which is a curated high-resolution, accurate mass spectral database. Compound identifications improved with increasing resolution but at a lesser extent than that of feature detection. Our findings suggest that mass spectral resolution higher than 60,000 is essential for obtaining greater metabolome coverage.

P-67	Metabolite profiling and bioactivity evaluations for the evaluation of family specific bioactive compounds for Cornaceae, Fabaceae, and Rosaceae families

PRESENTING AUTHOR: *Su Son, Konkuk University, South Korea*

CO-AUTHORS:

Thirty-four species from three plant families, namely Cornaceae (7), Fabaceae (9), and Rosaceae (18) were subjected to metabolite profiling using gas chromatography–time-of-flight-mass spectrometry (GC–TOF–MS) and ultrahigh performance liquid chromatography–linear trap quadrupole-ion trap-mass spectrometry (UHPLC–LTQ-IT-MS/MS), followed by multivariate analyses to determine the metabolites characteristic of these families. The partial least squares discriminant analysis (PLS-DA) revealed the distinct clustering pattern of metabolites for each family. The pathway analysis further highlighted the relatively higher proportions of flavonols and ellagitannins in the Cornaceae family than in the other two families. Higher levels of phenolic acids and flavan-3-ols were observed among species from the Rosaceae family, while amino acids, flavones, and isoflavones were more abundant among the Fabaceae family members. The antioxidant activities of plant extracts were measured using ABTS, DPPH, and FRAP assays, and indicated that extracts from the Rosaceae family had the highest activity, followed by those from Cornaceae and Fabaceae. The correlation map analysis positively links the proportional concentration of metabolites with their relative antioxidant activities, particularly in Cornaceae and Rosaceae. This work highlights the pre-eminence of the multi-parallel approach involving metabolite profiling and bioactivity evaluations coupled with metabolic pathways as an efficient methodology for the evaluation of family specific bioactive compounds.

P-68	An Untargeted Metabolomics Approach to Using High Resolution Mass Spectrometry for Identifying Disease Biomarkers

PRESENTING AUTHOR: *Gina Tan, Thermo Fisher Scientific, United States*

CO-AUTHORS: *Svetlana Rezinciuc, Heather Smallwood, Andreas Hühmer*

Metabolomics has increasing importance in biomarker discovery as detection and quantification of metabolites offers complementary value to genomics and proteomics studies. Observing metabolic changes occurring alongside gene alterations or protein activity can provide deeper understanding of a disease occurrence. Metabolite identification and profiling in biological samples however are challenging due to physiochemical diversities and varying abundance levels. To address these challenges, we pursue the LC-MS analytical approach using high resolution mass spectrometry and comprehensive software tools. Greater identification accuracy and confidence can be achieved and this workflow could potentially be applied onto clinical biological samples analysis. Nasal cell pellets were extracted from patient nasal wash samples through washing procedures. Metabolite extraction was performed and samples were filtered to remove salt content. Analysis was on a Thermo Scientific™ Orbitrap Fusion™ Tribrid™ mass spectrometer in ESI positive polarity at 120K resolution using a fine-isotopic determination data-dependent MS2 method. A 300ul/min flowrate, 15 min LC gradient with a reverse-phase C18 column chromatography method was used and data processing was on Thermo Scientific™ Compound Discoverer 2.0 software. Compound identification was done through automated mzCloud™, Chemspider searches, and KEGG pathway mapping. We identified 459 compounds by using a highly curated repository during the identification process. This untargeted LC-MS approach was advantageous because the high resolution MS system and intuitive statistical software capabilities assisted the detection of metabolites. These initial results are a proof-of-concept for similar biomarker discovery studies and present the ability to conduct further metabolite profiling studies with targeted metabolite panels for assay studies.

P-69	Large-Scale Generation of CCS Values to Support Unambiguous Identification of Metabolites in Untargeted Metabolomics and Lipidomics

PRESENTING AUTHOR: *Zhengjiang Zhu, Chinese Academy of Sciences, China*

CO-AUTHORS: *Zhiwei Zhou*

In untargeted metabolomics, unambiguous identification of metabolites remains a major analytical challenge. The use of collision cross-section (CCS) values of metabolites derived from ion mobility-mass spectrometry (IM-MS) effectively increases the confidence of metabolite identification, but this technique suffers from the limit number of available CCS values. Recently, we developed a machine learning based algorithm to accurately predict metabolites' CCS values using molecular descriptors. The prediction method was externally validated with a median relative error of ?3%. Using this method, we generated a large-scale predicted CCS database, namely, MetCCS, containing 35 203 metabolites. For each metabolite, CCS values for five ion adducts in positive and negative modes were predicted, accounting for 176,015 CCS values in total. MetCCS is freely available on the Internet (http://www.metabolomicsshanghai.org/MetCCS). Common users with limited background on bioinformatics can benefit from this software and effectively improve the metabolite identification. Users can identify unknown metabolites using experimentally measured m/z and CCS values within the defined tolerance. More recently, we further applied the developed prediction method to generate the CCS values of lipids. About 300 CCS values for lipids were experimentally measured and used as training dataset. We optimized 49 out of 286 molecular descriptors of each lipid for the prediction. The prediction accuracy for lipid CCS values has been largely improved with a median relative error of ~1%, better than the prediction of metabolite CCS values. Finally, we demonstrated that the addition of CCS values in untargeted metabolomics and lipidomics workflow could significantly improve the identification accuracy and selectivity.

P-70	Subcellular metabolite tagging via biochemical derivatisation with nitroreductase

PRESENTING AUTHOR: *Thomas Kralj, Monash University, Australia*

CO-AUTHORS: *Daniel Priebbenow, Anubhav Srivastava, Ghizal Siddiqui, Rodrigo Morales, Jonathan Baell, Darren Creek*

The application of metabolomics is helping to develop a deeper, mechanistic understanding of cellular biochemistry and the development of novel metabolomics methods offers more versatile and targeted methods to do so. Determination of organelle-specific localisation of certain metabolites can offer greater insight into the metabolic function of biological systems at the subcellular level. However, current metabolomic techniques for the determination of subcellular metabolite localisation and quantification lack efficiency, specificity and are prone to contamination. In this study, a novel method was investigated for derivatising subcellular metabolites present in the mitochondria by using a mitochondria-targeted nitroreductase (TAT-mMDHts-NfsA-GFP) enzyme coupled with nitro-aromatic derivatising probes. It was demonstrated that NfsA could tag metabolites containing carboxyl or thiol groups by converting nitro-aromatic probes into nitrogen radicals, producing derivatised metabolites with a signature mass shift detectable by mass-spectrometry. While the enzyme is capable of cellular penetration, transport into the mitochondria was not achieved, likely due to the pore size of the mitochondrial import channel. This study illustrates the potential for novel methods of subcellular metabolomics via metabolite derivatisation; although, further investigation is required into more versatile nitro aromatic probes and methods of organellar localisation.

P-71	Serum metabolomics reveals the treatment effect of the water extract of Polygoni Cuspidati Rhizoma et Radix in hypercholesterolemic rats

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CO-AUTHORS: *Tung-Ting SHAM, Sibao CHEN, Shun-Wan CHAN, Daniel Kam-Wah MOK*

This study aims at using untargeted metabolomics approach to examine the hypolipidemic function of the water extract of a traditional Chinese medicine, Polygoni Cuspidati Rhizoma et Radix (PCRR) which is known as "Huzhang" in Chinese, on high fat diet (HFD)-induced hypercholesterolemic Sprague-Dawley rats by Ultra-Performance Liquid Chromatography Quadrupole Time of Flight-Mass Spectrometry (UPLC-QTOF-MS). The major components of PCRR water extract have been identified as polydatin, emodin, resveratrol, emodin 8-O-?-D-glucopyranoside and physcion O-?-D-glucopyranoside using HPLC-DAD. The HFD-fed rats taking the PCRR extract supplement (450 mg/kg/day, p. o.) showed significantly lower levels of serum and liver total cholesterols. In the partial least squares-discriminant analysis of the UPLC-MS data, the cluster of PCRR-treated group was well separated from those of normal control or HFD model in the score plot. This indicated that the serum metabolite profiles of the PCRR group was very different from the other groups. Multivariate statistics revealed that treatment of PCRR to hypercholesterolemic rats resulted in significantly elevated levels of circulating chenodeoxycholate, deoxycholate, their glycine-conjugated bile acids and glycocholate level and restored tryptophan and lysophosphatidylethanolamine 20:4 levels when compared with HFD model. These results indicated that the underlying cholesterol-lowering mechanism of PCRR might be associated with bile acid biosynthesis. Besides, a number of resveratrol-related metabolites via microbial transformation were solely identified in the serum of PCRR-treated group. We believed that treatment of PCRR could modulate the structure of gut microbiota during intervention.

P-72	Profiling of Wine using ultra-high resolution Flow Injection mass spectrometric Analysis and 1H-NMR Spectroscopy

PRESENTING AUTHOR: *Chris Thompson, Bruker, United States*

CO-AUTHORS: *Matthias Witt, Nikolas Kessler, Markus Godejohann*

The complexity of organic compounds in food products such as wine can be analyzed by mass spectrometry. Beside GCMS and LCMS, wine can be analyzed on the molecular level by Flow Injection Analysis (FIA) after solid phase extraction (SPE) when combined with ultra-high resolution mass spectrometry. The mass spectra are a fingerprint of these complex mixtures of organic compounds. Therefore, wines can be differentiated very quickly on the molecular level using this mass spectrometric technique. Multivariate statistical analysis of FIA-MS and 1H-NMR spectroscopy resulted in similar results. Wine samples were analyzed after solid phase extraction (SPE) using a Bruker solariX XR 7T mass spectrometer using ESI (-) with a resolving power of 300,000 at m/z 400. Mass data were subjected to deisotoping and adduct collation. Statistical analysis such as PCA and HCA as well as molecular formula calculation based on accurate mass, isotopic fine structure and filtering based on elemental composition was carried out automatically in MetaboScape 3.0. Annotated features were investigated using filters for mass defects and DBE. SPE wine extracts were subjected to a Bruker FoodScreenerTM (400 MHz Avance III NMR spectrometer). Automatic solvent suppression of the solvent resonances enabled the detection of organic constituents present in the extracts. Wine samples were measured in replicates to check reproducibility of the results for the multivariate statistical analysis using Principle Component Analysis (PCA). The results of both multivariate statistical analyses (FIA-MS and 1H-NMR) were very similar: Bordeaux wine were separated from the red and white wines from Loire and Alsace.

P-73	Shaofu Zhuyu Decoction Ameliorates Obesity-mediated Hepatic Steatosis and Systemic Inflammation by Regulating Metabolic Pathways

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CO-AUTHORS: *Moonju Hong, Hee-Sook Park, So Min Lee, Soon-Hee Kim, Kyung-Won Lee, Nam-Joo Jeong, Myung-Sunny Kim*

The pathogenesis of hepatic steatosis is related to blood stasis syndrome (BSS) in traditional Korean medicine (TKM) and Chinese medicine (TCM). In this study, we evaluate the treatment effects of Shaofu Zhuyu decoction (SFZYD), one of the famous BSS treatment remedies, on high fat diet (HFD)-induced inflammation and hepatic steatosis. The metabolic profiles of sera obtained from normal chow diet (NC) group, a HFD group, and a SFZYD-treated DIO group were investigated using CE-MS spectroscopy coupled with multivariate statistical analysis. Histological and biochemical examinations indicated that SFZYD treatment ameliorates hepatic steatosis and systemic inflammation. In score plot of partial least squared – discriminant analysis (PLS-DA) from the sera showed a metabolite shift in the HFD mice toward the NC group after SFZYD administration. The significantly altered metabolites represented that SFZYD regulated energy metabolism, the pentose phosphate pathway and aromatic amino acid metabolism. In addition, SFZYD treatment recovered the expression of genes related to inflammation, e.g. Ccl2, Tnf?, Il1?, Il6, Serpine1, AdipoQ in the liver and adipose tissue. Furthermore, correlation network showed that metabolic alteration by SFZYD significantly related to the blood parameters and expression of genes, related to inflammation, lipid metabolism, and mitochondrial dysfunction. These results suggest that SFZYD may ameliorate HFD induced hepatic steatosis and inflammation by regulating the related metabolism and biomarkers.

P-74	Metabolomic heatmaps as a tool for strain prioritization in natural product research

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CO-AUTHORS: *Michael Marner, Jens Glaeser, Armin Bauer*

Metabolomic heatmaps as a tool for strain prioritization in natural product research The continuous demand for new antibiotics to overcome evolved resistance led to the extensive exploration of known antibiotic producing bacterial phyla, such as Actinobacteria, leading to high rediscovery rates. Thereby, innovative cultivation techniques and the investigation of underexplored phyla represent promising approaches to discover novel antibiotics.1,2 However, accessing new bio resources provide a large amount of poorly characterized isolates for further analysis. Next, the similarity of the individual extracts is calculated based on this peak table. Successive clustering and plotting is performed using a custom R script and results in heatmaps. This provides a visual way to investigate the cultured diversity on a metabolomic level. 3To prioritize new isolates based on their chemical profile, we implemented a metabolomic heatmap approach. First, the data is processed by XCMS and a peak table is created. Using this approach we could demonstrate that phylogenetic closely related strains cluster together based on their metabolomic similarity. This approach helps to focus on a larger chemical diversity for downstream processes and further analytics. Also, analytics of strains that show a very high metabolomic similarity can be limited to one representative. All in all this strategy might increase chances to find new chemistry in underexplored biological niches that could ultimately be a new lead structure for further pharmaceutical development.

P-75	Specific Detection of Cellular Glutamine Hydrolysis in Live Cells Using HNCO Triple Resonance NMR

PRESENTING AUTHOR: *Xing Jin, Seoul National University, South Korea*

CO-AUTHORS: *Sujin Lee, He Wen, Jin Wook Cha*

Glutamine plays key roles as a biosynthetic precursor or an energy source in cancers, and interest in its metabolism is rapidly growing. However, the proper evaluation of glutamine hydrolysis, the very first reaction in the entire glutaminolysis, has been difficult. Here, we report a triple resonance NMR-based assay for specific detection of glutaminase activity carrying out this reaction using stable-isotope labeled glutamine. Compared to conventional methods involving coupled enzyme assays, the proposed approach is direct because it detects the presence of the H?N?CO amide spin system. In addition, the method is unique in enabling the measurement of glutamine hydrolysis reaction in realtime in live cells. The approach was applied to investigating the effects of a glutaminase inhibitor and the inhibitory effects of glucose on glutamine metabolism in live cells. It can be easily applied to studying other signals that affect cellular glutamine metabolism.

P-76	Using trapped ion mobility MS to separate isomeric sphingosines

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CO-AUTHORS: *Dirk Wunderlich, Verena Tellström, Niels Gödecke, Oliver Räther*

Galactosylsphingosine and glycosylsphingosine are important components in muscle and nerve cell membranes. Both belong to the class of cerebrosides and consist of the same lipid residue with a different carbohydrate moiety: Galactose vs. Glucose. They are involved in several diseases. Since they have the same elemental composition and differ only by the orientation of a hydroxyl group, they are structural isomers. They cannot be distinguished from each other in a mixture just by exact mass measurements. An HPLC separation is challenging as the structural difference is very small. Here we report on the use of high resolution ion mobility MS to unambiguously separate these two compounds. Several adducts were investigated for their potential to separate these compounds with trapped ion mobility mass spectrometry. Ions generated from salts forming [M+H]+, [M+Na]+, [M+Li] were not useful for ion mobility separation. Interestingly, the silver adducts gave a clear difference in the 1/k0 values of the two analytes allowing baseline separation in a mixture. A high ion mobility resolution of > 200 provided by the timsTOF could be achieved and is necessary for successful separation. A simulation of lower ion mobility resolutions of 60 or 100 showed that they would be not sufficient to resolve the two sphingosines in the mobility dimension from each other. Baseline separation requires a resolution of >200 which was achieved for the silver adducts. Furthermore collision cross section (CCS) values were determined for the silver adduct ions as well as for all other ions.

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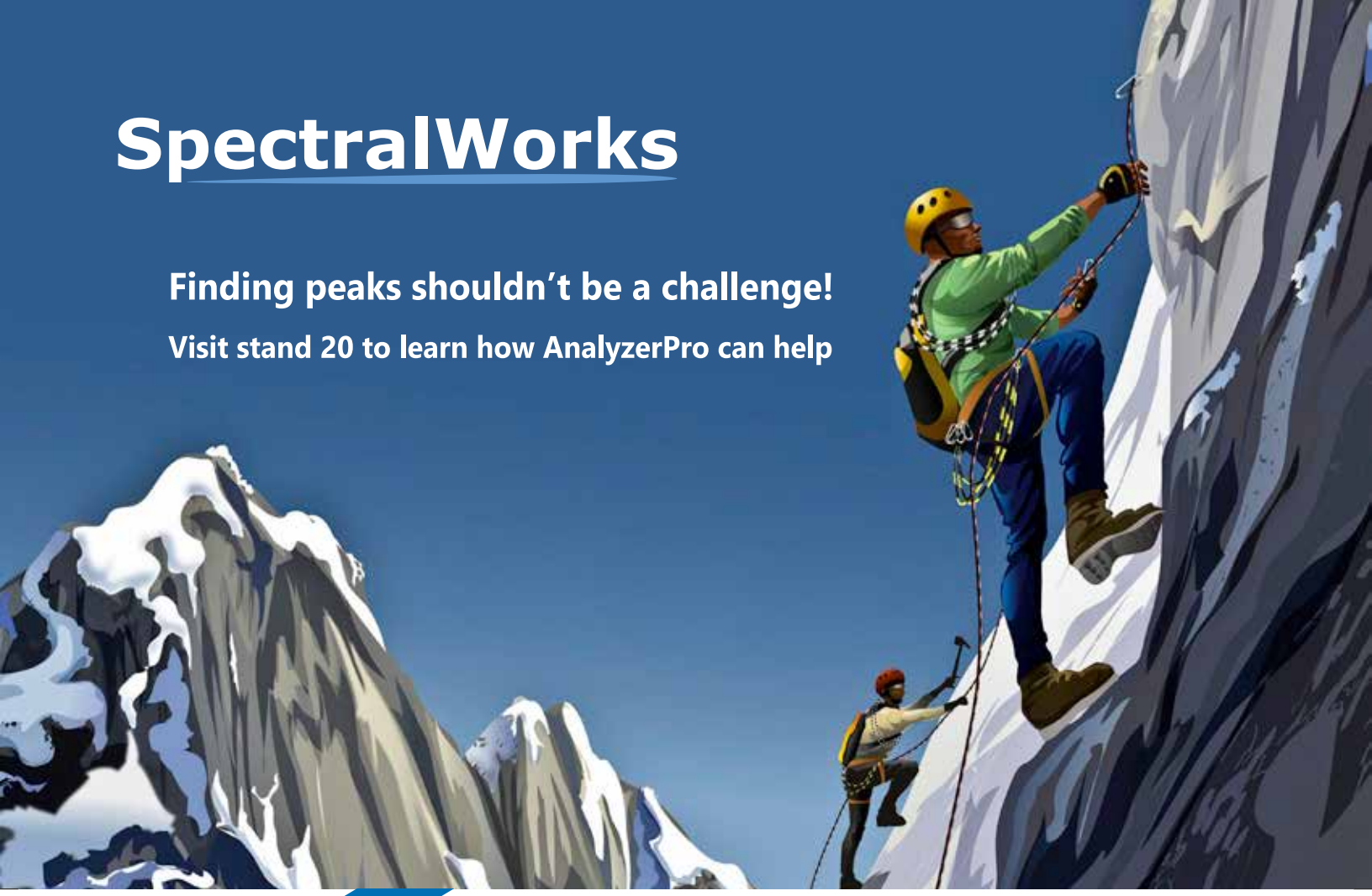

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P-77	Influence of bioactive compounds of milk on intestinal cell cultures

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CO-AUTHORS: *Tobias Demetrowitsch, Ingrid Clawin-Rädecker, Martin Klempt, Silke Szymczak, Stephan Seifert, Karin Schwarz*

Introduction: Milk- and galacto-oligosaccharides (MOS/GOS) are natural compounds in mammalian milk. It is quite known that MOS/GOS have bio-functional properties like prebiotic, immune modulating, bifidogenic and anti-inflammatory effects, but the mechanism is unknown. The aim of this study was to analyse the effects of MOS/GOS on the lipid- and metabolome of intestinal cells. In addition, specific metabolic patterns and corresponding pathways should be identified. Material and Methods: The experimental protocol was a cell treatment for 24 h with different compositions of MOS or GOS. The cell-cultivation followed a harvest by physical force and acidified water, cell disruption with ultrasonic on ice and finally a SIMPLEX extraction. SIMPLEX allows the simultaneous extraction of metabolites, proteins and lipids. The samples were analysed by shotgun ESI-FT-ICR-MS. Data validation was conducted by the quality control (QC) approach (Demetrowitsch et al., 2015) and were evaluated by non-targeted (supervised and non-supervised) and targeted approaches. Results: First results from the non-supervised approach provides a correlation between treatment and cell metabolome. The PCA-models showed tight clusters for each treatment. Significant regulations were found e.g. for the compounds 543.1334 [M+K] with the calculated formula C19H28N4O12 and 279.0391 [M+Na] with the calculated formula C11H12O5P. The targeted lipidomics approach showed significant effects between the treatments. Highly significant changes were analysed for e.g. phosphatidylinositol (42:3), phosphatidylcholine (22:4) and phosphoglyceride (28:5).

P-78	Metabolic reaction network based metabolite annotation in untargeted metabolomics

PRESENTING AUTHOR: *Xiaotao Shen, Chinese Academy of Sciences, China*

CO-AUTHORS: *Zheng-Jiang Zhu*

In untargeted metabolomics, LC-MS has enabled the detection of thousands of metabolite peaks. However, the metabolite identification remains the major bottleneck. MS/MS spectrum matching is the most widely used method for metabolite annotation, but is largely limited by the number of available MS/MS spectra. Here, we developed a recursive annotation workflow which uses identified metabolites as seeds and integrates metabolic reaction network to annotate metabolites. Specifically, we first created an in-house library containing more than 800 metabolite standards with measured MS/MS spectra and retention time (RT). A metabolic reaction network containing 8,074 metabolites and 15,826 reaction pairs was constructed. For metabolites in the reaction network, a machine-learning based prediction model was developed to predict their theoretical RTs using 800 standards as the training dataset. Secondly, the detected metabolic peaks were matched to our in-house library for identification. Roughly 100-200 metabolites were identified in common biological samples. Thirdly, the identified metabolites are used as seeds to annotate other detected metabolic peaks without a match with our in-house library. Peaks are checked whether they are isotopes or adducts of seed metabolites. Then, metabolic peaks that have high intensity correlation and high structure similarity with seed metabolites are further annotated using the metabolic reaction network. Furthermore, the putative annotated metabolites are scored and cross-validated to determine a cutoff score. Finally, new annotated metabolites are used as new seeds for next round annotation. The whole dataset are annotated using this recursive workflow until all the peaks are annotated.

P-79	Targeted and untargeted multivariate comparison of LCMS data from New Zealand propolis and its likely poplar sources

PRESENTING AUTHOR: *Kevin Mitchell, Callaghan Innovation, New Zealand, New Zealand*

CO-AUTHORS: *Stephen Bloor, Rosemary Webby, Trevor Jones*

New Zealand propolis is known to be a poplar type propolis. Poplar trees are widely planted in the NZ rural environment for shelter and erosion control and many specialised hybrid varieties have been bred for this purpose and are unique to NZ. We are undertaking a study of the possible poplar sources in the New Zealand environment. For this comparative work, we carried out both untargeted data analysis using low resolution mass accuracy UPLC-MS data and a more targeted study of metabolites from a reduced sample set collected from a high mass accuracy UPLC-MS system. Samples of NZ propolis were obtained from several NZ localities as well as several commercially produced products (pooled localities). A range poplar leaf and bud extracts were collected from the various poplar cultivars under examination. The targeted study was limited to compounds we could identify and a number of unidentified compounds commonly occurring in the dataset. Raw data from both LCMS datasets were initially processed using MZMine to detect and integrate peaks eluting in each LCMS run, and to align chromatograms and generate grouped peak tables. A range of multivariate techniques, including principal components analysis, multidimensional scaling, and neural networks etc., were applied to these two datasets (using R libraries) to try to identify which varieties(s) of poplar were most similar to raw propolis. The objective of this study is to assist plantsmen in planting bee friendly varieties of poplar and help apiary based industries to determine which poplar varieties produce the highest quality propolis.

P-80	A metabolomics approach towards understanding the aetiology of Acute Bovine Liver Disease

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CO-AUTHORS: *Mark Hawes, Grant Rawlin, Simone Rochfort*

Acute Bovine Liver Disease has been recognised for decades as a threat to cattle in the South East of Australia, mainly occurring in parts of Victoria and Tasmania. Its sporadic occurrence has hindered efforts to identify and control the cause even though it is associated with Rough dog's tail (RDT), a grass that is usually found in 'toxic' paddocks as old, dry forage. Previous investigations into the toxicity of RDT have shown that it is non-toxic to cattle two months after the danger period for ABLD. Investigation into fungal species found on RDT at the time of harvesting has identified a species of Drechslera as a possible culprit. However, the toxin that causes ABLD and its origins are currently unconfirmed. In order to identify a biochemical toxin fingerprint, and potentially identify the toxin, a metabolomics approach has been utilised to investigate the disease. Liver and urine samples have been collected from healthy cattle and cattle that have succumbed to the toxicity. Aqueous extracts were analysed using a Q Exactive mass spectrometer and a 700 MHz NMR. Initial statistical investigation showed sample clustering according to disease state. Potential biomarkers of disease have been found, many of which remain, as yet, chemically unidentified. Comparison with forage and fungal metabolomes will be discussed. The identification of common features between the plant or fungal metabolomes with ABLD animals will allow the identification of exotic metabolites in the animal tissues. These common features may be candidate toxins and strategies towards compound identification will be discussed.

P-81	Data Independent Analysis: An appropriate tool for qualitative and quantitative metabolomics?

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The structural annotation or identification of metabolites in untargeted metabolomics studies is a key process to derive biological knowledge. When applying UPLC-MS, chromatographic retention time/accurate mass /gas phase fragmentation data are collected to aid metabolite identification. The interrogation of full scan data to derive molecular formula is routinely applied [1]. The collection of MS/MS data to derive structural information is also applied. Data Dependent Analysis (DDA) is traditionally applied and provides high specificity but low coverage of metabolites with MS/MS data. Data Independent Analysis (DIA) sequentially acquires MS/MS data for all metabolites in multiple DIA m/z windows (typically of m/z width 25-50). This approach provides a reduced specificity but 100% coverage of metabolites with MS/MS data. Deconvolution after data acquisition increases specificity [2]. DIA is not routinely applied in metabolomics. We will describe a robust characterisation of DIA for metabolite identification and absolute quantification on Thermo Scientific Q Exactive instruments coupled to UPLC separations. We have assessed DIA window width, mass resolution and data processing software for different UPLC separations (HILIC, RP, lipidomics) and sample types (urine, plasma, mammalian tissue). We have shown that data is not as complex as expected which allows larger DIA windows and a higher mass resolution to be applied. We have also demonstrated how DIA improves the number of metabolite identifications in an untargeted study and how DIA can be applied for quantitation analysis. [1] Brown, M., et al. Bioinformatics, 2011, 27(8), 1108-1112. [2] Tsugawa, H., et al. Nature Methods, 2015, 2(6), 523-526.

P-82	METABOLIC REGULATORY VARIATIONS IN RATS DUE TO CHRONIC COLD STRESS: HIGH RESOLUTION 1H NMR APPROACH

PRESENTING AUTHOR: *SONIA GANDHI, INSTITUTE OF NUCLEAR MEDICINE & ALLIED SCIENCES (INMAS), DRDO, India*

CO-AUTHORS: *SUNIL KOUNDAL, SUBASH KHUSHU*

Thermal conditions influence the development of living organism in a wide variety of ways triggering various adaptive responses. Evidence exist for the adverse effects of chronic cold stress on human health including rapid loss of homeostasis & long term exposure might result in cardiovascular & respiratory diseases like hypertension & asthma, diseases relating to immune system, diarrhea. NMR spectroscopy in conjugation with statistical analysis such as Principal Component Analysis (PCA) can be successfully used as a non-invasive tool to identify biomarkers for an early biochemical changes induced due to cold exposure. Chronic cold exposure on rats at 4C for 14 days (8hrs each day) showed perturbations in endogenous metabolites such as as citrate, 2-oxoglutrate, succinate, fumarate, Creatinine, creatine & taurine in rat urine samples pre & post exposure. These changes were indicative of altered energy metabolism, altered glomerular filtration rate or gut microbiota & altered liver functioning. The present study indicates NMR based metabonomics in conjugation with statistical analysis serves as a powerful tool for non-invasive monitoring & early predictive markers of various metabolic alterations in urine induced by prolonged cold stress. These studies in correlation with in vivo imaging, biochemical parameters can form strong basis for mass screening & developing strategies to combat cold stress.

P-83	The study of plasma metabolite profile after administration of Harak Formula (HRF) in relation to platelet aggregation in individual healthy volunteer

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Harak Formula (HRF) is a polyherbal formulation. It has been used in Thai traditional medicine as a remedy for fever. HRF is a combination of five dried roots powder of herbal plants with equal amounts. Phytochemicals probably consist of multiple active components that are responsible for pharmacological activities. Due to the lack of their pharmacokinetic data in human. Our study, therefore, applied a metabolomics-based mass spectrometry to profiling metabolites in HRF that affect platelet aggregation. Five healthy volunteers (2 males and 3 females) which ages between 29-40 years were recruited into this study. All subjects have received a single dose of HRF at the maximum recommended dose per day (1,500 mg) according to the latest Thai National List of Herbal Medicinal products. Platelet aggregation was measured by aggregometer at pre-dose and 3, 6 and 24 hours after HRF administration. Although, variation in platelet aggregation was seen in some individuals with specific agonist. The average percentage of platelet aggregation revealed no significant difference at any time points during the study. Identification of metabolic profile using LC-QTOF was performed in plasma at pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6 and 24 hours after dose. Untargeted metabolomics revealed changes in metabolic profile during 1-3 hours after HRF administration. Interestingly, we suspected groups of phytochemicals that might be associated with alteration of platelet aggregation in the specific individual. Using integrative analysis, we found additional factors that affected platelet aggregation such as platelet status, sex, age and lipid profiles.

P-84	Variation in composition of Red Brazilian Propolis: seasonality vs. location.

PRESENTING AUTHOR: *Alexandra Sawaya, UNICAMP, Brazil*

CO-AUTHORS: *Begoña López*

In tropical countries, bees collect resins for propolis throughout the year; therefore season variations in composition are to be expected. Red propolis from the northeast of Brazil comes from native forests near the beach and mangrove areas, and its composition may also be affected by the vegetation in the region where it was collected. Studies comparing the composition of samples from different States (Alagoas, Paraíba, Espirito Santo and Bahia) and samples collected monthly over one year from Sergipe were compared by UHPLC-MS. Furthermore the concentrations of 9 components were quantified in all samples by a validated MRM method, by comparison to external calibration curves of isolated compounds or analytical standards. The results show a quantitative variation of these components throughout the year, and do not display a clear seasonal pattern, indicating that other factors (rain or drought) possibly affected the composition more than the time of year. The samples collected in other states of the northeast presented a similar qualitative composition, but with a wide variation in the concentration of individual components, even in samples collected within the same region. Several factors interfere in the chemical composition of red propolis: region, climate, time of the year. In order to use red propolis for its nutraceutical properties, further studies to determine ranges of concentration of these compounds, should be undertaken.

P-85	NMR metabolomics of infected poultry treated with a Campylobacter jejuni N-glycan based vaccine

PRESENTING AUTHOR: *Goncalo Gouveia, UGA-CCRC-EdisonLab, United States*

CO-AUTHORS: *Harald Nothaft, Arthur Edison, Christine Szymanski*

Poultry meat is considered to be the primary source of human Campylobacter jejuni infection, therefore there is strong interest in a cost-effective solution to reduce C. jejuni levels in poultry. Vaccination is a promising public health strategy. Oral administration of an attenuated Escherichia coli strain engineered to express the antigenic Campylobacter jejuni N-glycan on its surface was shown to reduce C. jejuni colonization in leghorn and broiler chickens and thus has a great potential to prevent pathogen entry into the food chain. However, the birds demonstrated a bimodal response to this vaccination as well as differences in their respective microbiota. In addition, broilers vaccinated with or without Anaerosporobacter mobilis, a novel probiotic, and with or without Lactobacillus reuteri, a commonly used probiotic, demonstrated that probiotic addition results in increased vaccine efficacy, vaccine compound-induced immune responses, and weight gain. Here we describe an untargeted NMR-based metabolomics approach to complement and further expand on these findings. Cecal samples from five experimental groups 1) negative control, 2) infected but no vaccine control, 3) vaccine, 4) vaccine + L. reuteri and 5) vaccine + A. mobilis were analyzed by 1D 1H NMR. We identified NMR features correlated with C. jejuni infection that do not have clear database matches. We are applying 2D NMR, HPLC-SPE and MS to identify these features and further investigating the cecal metabolome for additional metabolites that significantly differ across experimental groups and between vaccine responders and non-responders.

P-86	Citrus volatile and non-volatile cues as putative kairomones for Diaphorina citri

PRESENTING AUTHOR: *Carmen Rossini, Facultad de Química, Universidad de la Republica, Uruguay*

CO-AUTHORS: *Anna Burgueño, María Amorós, Virginia Pereira Das Neves, José Buenahora, Andrés López, Guillermo Moyna*

Huanglongbing, the most destructive citrus disease worldwide, is dispersed by the Asian citrus psyllid (ACP), Diaphorina citri. During host plant search, ACP integrates information from shoots including visual and odor cues. To characterize oviposition preferences of ACP a greenhouse experiment using 6 citrus species was carried out. Results showed that ACP exhibits a preference for 3 species. Elemental analyses on shoots from the 6 species showed no significant diferences correlated to the oviposition preference. Therefore we have searched for markers that may point to putatitve kairomones in the volatile organic compounds (VOC) and the leaf waxes (LW) by GCMS and in CDCl3 and D2O extracts by NMR. GCMS data from VOC were conventionally analyzed, and GCMS from LW were analyzed using MZmine2.10, defining parameters for mass detection, chromatogram building and peak deconvolution. Normalized chromatograms were then aligned, generating a matrix of 646x15. NMR data were processed with MestReNova 11.0 (0.04 binning; 11-0 ppm) generating matrices of 260x12 and 247x12 for CDCl3 and D2O extracts respectively. All data sets were then subjected to multivariate analyses (PCA, PLS , OPLS). In the case of CDCl3 extracts, the chemical profiles of the preferred citrus species were more similar among them that the ones not-preferred, pointing to non-polar compounds as possible cues for ACP. The analyses on VOC also grouped preferred species together. This grouping could be traced to the presence of methyl-N-methylanthranilate. Our results suggest that a combination of long-range and contact cues could be correlated with the observed ACP oviposition preference.

P-87	1D and 2D NMR approaches to covering the metabolome of soybean upon abiotic stress

PRESENTING AUTHOR: *Isabel Coutinho, Embrapa Instrumentação, Brazil*

CO-AUTHORS: *Liliane Henning, Alexandre Nepomuceno, Christian Richter, Harald Schwalbe, Luiz Colnago*

In this work, we explored the feasibility of 1D and 2D NMR spectroscopy to identify metabolic changes in soybean leaves and roots subjected to water-deficient and waterlogging conditions. The putative metabolite annotation using 1H 1D and 2D NMR spectroscopy led to identification of 33 primary metabolites and 13 secondary metabolites in soybean leaf and root. Multivariate analysis of the 1H NMR data from control and waterlogging soybean leaves and roots extract revealed two cluster according to primary metabolites alanine, citric acid, malic acid, succinic acid, GABA and secondary metabolites. Tolerant genotype does not accumulated higher concentration of alanine and TCA metabolites in roots under flooding conditions when compared to sensitive ones. The sensitive genotype accumulated more kaempferol and cafeoyl derivatives than tolerant genotype. In addition, 1D PSYCHE* and 2D NMR experiments were performed to improve resolution and accurate metabolite annotation. The utility of 1D PSYCHE is demonstrated in a study of involving comparative metabolomics of soy genotypes tolerant and sensitive to water deficit. *Foroozandeh, M., Adams, R. W., Meharry, N. J., Jeannerat, D., Nilsson, M., Morris, G. A. Ultrahigh-Resolution NMR Spectroscopy. Angew. Chem. Int. Ed., 53, 6990-6992, 2014.

P-88	The Challenges of Identification - What 'Identifies' a Compound

PRESENTING AUTHOR: *Tim Stratton, Thermo Fisher Scientific, United States*

CO-AUTHORS: *Robert Mistrik*

The identification of compounds detected in biological sample analysis is one of the single largest challenges in metabolomics. The confident identification of both previously known compounds or the elucidation of relatively or completely unknown compounds requires sufficient information about the compound of interest to provide a robust confidence in the assigned identification. Several excellent publications have proposed structures to build scoring systems to specify differing and increasing levels of confidence or support for an identification. In the context of these, we will discuss the various degrees of information that mass spectrometry alone can provide and the relative 'value' of the identifications that can be assigned based on this data. Semi-targeted techniques, to confirm the identification of previously identified compounds, will be differentiated from 'true unknown' approaches. In addition, consideration will be paid both to the ability to determine elemental composition and utilize this for various database lookup approaches as well as fragmentation spectral based identification through spectral matching or substructure identification. In our presentation, special attention will be paid to spectral library techniques – both for the identification of previously known compounds through either MS/MS or MSn means, and the capability of identifying previously unknown compounds through substructure identification utilizing information from previously known compounds.

P-89	QUINOLIZIDINE-TARGETED ANALYSIS OF FABACEOUS INVASIVE PLANTS FROM WILD AND PROPAGATED ACCESSIONS

PRESENTING AUTHOR: *Ericsson Coy-Barrera, MU Nueva Granada, Colombia*

CO-AUTHORS: *Willy Cely-Veloza, Ana Romero-Rincón*

Fabaceae family is a big group of flowering plants. Several of them have been introduced in various countries for diverse purposes but some become a problem because of biological invasions. In Colombia, two Fabaceous species were introduced from Europe for ornamental uses such as Ulex Europeaus and Genista monspessulana, and they have progressively dominated some native environments as well as altering many aspects of ecosystem functioning. Other invasive-behaved Fabaceae ones are native such as some Lupinus species. For invasion success, this kind of plants can produce bioactive secondary metabolites such as quinolizidine-related compounds. Thus, as part of our research on chemoprospecting of invasive plants, several plant accessions (n>60) of U. europeaus, L. bogotensis and G. monspessulana, from different invaded places in Bogotá plateu, were separately investigated through a comprehensive targeted GC-MS-based metabolomics approach from alkaloid-enriched extracts in order to observe the quinolizidine-based chemical variability between samples (ontogeny and environmental factors) and its implication on antifungal activity against F. oxysporum. MS-mediated annotation showed the occurrence of different sparteine and lupanine-like tetracyclic quinolizidines. Phytomaterials also showed antifungal capacity at different levels (2>IC50(µg/mL)>55). OPLS-DA-derived score plots indicated several differences between samples but clustered according characteristic chemical constituents and/or activity. Supervised analysis indicated the existence of three quinolizidine-related compounds to be responsible of the antifungal activity. The present targeted metabolomics exploration of these invasive plants is an excellent approach for quinolizidine-based antifungal finding from nature as well as ontogeny and environmental indicators (Present work was financed by UMNG through Project IMP-CIAS-2293 - Validity 2017).

P-90	Automation of sample preparation for metabolomic analysis using robotic platform

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Metabolomic analysis is prone to variation and errors due to manual processing at different states of sample preparation (e.g. extraction, purification, derivatization). Reproducible sample preparation is vital for ensuring comparable and reliable results for large sample series. It is important to develop methods for labor and time-saving sample preparation. We developed an automated sample preparation method using robotic platforms, which represent a modified Bligh and Dyer method producing samples for hydrophilic metabolomics by GC-MS and lipidomics by SFC-MS simultaneously. The first step of modified Bligh and Dyer method, the addition of 1 mL cold methanol:chloroform:water solution and internal standards for both GC-MS and SFC-MS analysis, was performed manually. Subsequent procedures were performed by the automated protocol using a PAL RTC System. Aqueous layer and organic layer were collected in clean glass vials after the automated Bligh and Dyer method. The glass vials containing the aqueous layers were transferred to a centrifugal vacuum concentrator for drying samples manually. Then, vials were transferred to another PAL RTC system for the automated just-in-time derivatization and injection system for GC-MS analysis. The vials containing organic layers were transferred to the SFC-MS autosampler and subjected to target lipidomic analysis. The precision of the relative peak area for each determined compound in the result of lipidomic and metabolomic analysis using the automated protocol was equivalent to that of the manual protocol by a skilled technician. The automated protocol facilitates the overnight extraction procedure and compound derivatization followed by on-line GC-MS analysis, resulting in significant labor-saving.

P-91	Database on Flavonoid in Korean Foods

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Flavonoids have received attention as functional ingredients because of their beneficial effects in the prevention of human diseases such as cancer and cardiovascular diseases. The health benefit of flavonoids is directly related to the daily dietary intake of antioxidants, it is important to evaluate flavonoid sources in food and to have overall information. Therefore, we have developed a comprehensive database on the nature and content of flavonoids contained in the main foods consumed by the diet. In total, 268 kinds of Agro-food samples were used for this database including cruciferous vegetables, and citrus such as grapefruits. Also, even though cereal crops, mushrooms and root and tuber crops such as potatoes rarely include flavonoids, 1~2 chromatograms are contained for each type. It included 3,205 quantitative values on a real standard substances basis and there are library containing 1,683 flavonoids, of which 846 were directly identified and quantitated. The analysis results contained in this database were obtained using UPLC-DAD/QTOF-MS combined with HPLC and mass spectroscopy. For quantitative analysis, HPLC peak areas were calculated using two types of specification internal standard materials. Isoflavone quantitation using fluorescein was measured at a wavelength of 254 nm, and flavonol, flavanol and chalcone were measured at a 280 nm using galangin, and flavonol and flavone were measured at 350 nm. This flavonoid database can be used as the basic data for the study and application of functional food ingredients to contribute to food industry development, understanding of metabolic pathways, and discovery of biological activity.

P-93	Detection and identification of opines in abalone adductor muscle: Theory meets practice

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Some marine invertebrates (like abalone) have the unique ability to produce opines as a means to prevent NADH accumulation during anaerobic conditions. Opines are produced by joining pyruvate with various amino acids at the expense of NADH. Despite all the information regarding this process and the presence of opines in abalone, identification of these compounds are complicated due to the lack of available standards and the fact that no opine MS/MS spectra and retention time information is available. In this study, we employed several techniques to obtain selective detection and confident identification of these compounds in abalone muscle. Metabolites were extracted from abalone adductor muscle, derivatised with butanolic hydrogen chloride and analysed using untargeted liquid chromatography quadrupole time-of flight-mass spectrometry. The theoretical masses, formulae and fragment patterns of butylated opines were used to detect and identify these compounds in abalone muscle. To achieve the highest level of confidence with our identification, we validated the predicted identities with in-house synthesised opine standards. We conclusively detected and identified the presence of alanopine, lysopine, strombine and tauropine as anaerobic end-products, produced following exposure to environmental hypoxia, in the adductor muscle of abalone.

P-288	Investigating MSMS coverage of a wheat profiling LC-MS data set using data dependant MSMS (ddMS2) and all ion fragmentation (AIF) strategies.

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Metabolite identification has long been a bottle-neck for metabolomics analyses. Unless comparing against an internal MS library, putative identification of metabolites can be difficult using mass of precursor ions in LC-MS acquisitions. As instrument capabilities improve, more features are being detected and mass spectral data for each feature is increasing. All ion fragmentation (AIF) and data dependant mass spectrometry (ddMS2) are two techniques for acquiring untargeted MSMS information using the Thermo Q-Exactive Orbitrap. AIF is a full scan MSMS technique, in which all molecules ionised in the source are further ionised in the C-trap, as opposed to ddMS2 whereby MSMS events of individual ions are triggered according to method criteria. Here, we examine the number and quality of MSMS features acquired using ddMS2 and AIF on pooled samples to investigate the coverage of features generated on a full scan only sample set. Eleven wheat varieties were profiled using full scan MS on a C18 column in positive ion mode. Three to four varieties were pooled to create three extracts that were analysed using identical LC and MS parameters as per the analytical samples, with the exception of ddMS2 and AIF specific parameters collected with a collision energy (CE) of 20eV. Both strategies supply analysts with spectral information previously only acquired by prior knowledge of analytes of interest. ddMS2 does not provide complete MSMS coverage, typically triggering on the most abundant precursor ions, whereas AIF can potentially supply a greater depth of information but at a potential issue to spectral purity.

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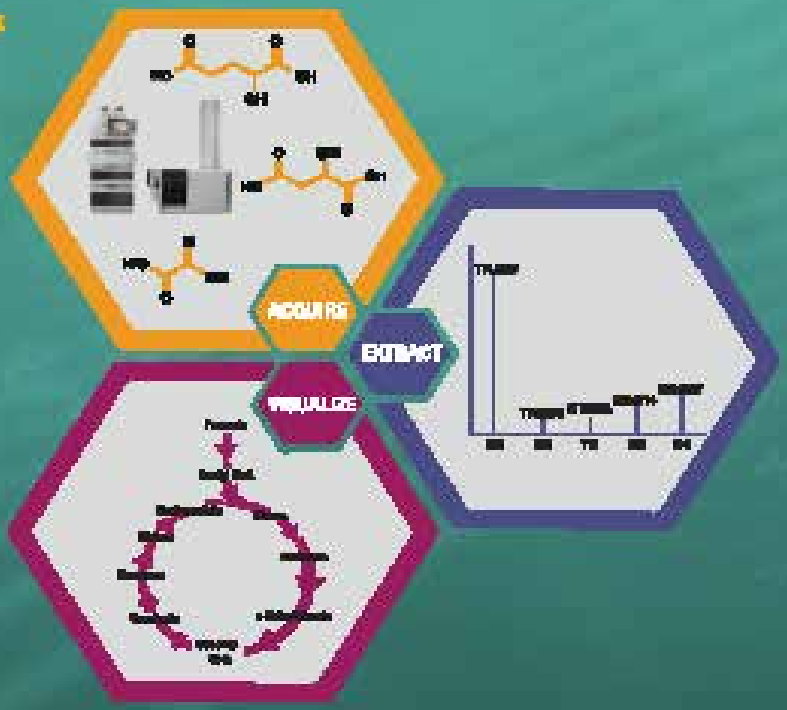

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O-139	Differential multi-fluid network sheds light on metabolic processes altered in end-stage renal disease

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Chronic kidney disease (CKD) is an increasing public health problem, affecting 14% of the Western populations. Here, we aimed to identify metabolic processes that account for the widespread metabolic shift commonly observed in renal disease. Metabolomic profiling was conducted on a non-targeted mass spectrometry-based platform for plasma, urine, and saliva samples collected at the same visit from 72 CKD patients (GANI_MED cohort) and 906 population controls (SHIP_TREND). After preprocessing and imputation of missing values, metabolite levels were corrected for age, sex, and BMI. Gaussian Graphical Models (GGMs) were inferred from the measured data to model metabolic processes within and between fluids in cases and controls separately. Differences between the two models were assessed by permutation testing. The multi-fluid GGMs consisted of 882 metabolites, connected by 4404 and 2509 edges for cases and controls, respectively. 86 edges were significantly different between the models, after correction for multiple testing, mainly highlighting two metabolic processes: (i) xanthine catabolism, suggesting a lack of detoxification and excretion of xenobiotics probably due to alterations of renal filtration; (ii) steroid metabolism, which can cause complications of CKD, including cardiovascular disease, due to its effect on hydro-electrolyte and glycaemic control, hypertension, and the immune system. This is the first study on renal disease integrating metabolomics data from three different fluids. Modelling metabolic processes across fluids using GGMs allowed to focus on disturbed metabolic processes rather than differences in metabolite levels between patients and controls. By highlighting key processes, our results illustrate the power of integrative, multivariate data analysis.

O-171	Laboratory Evolution Reveals Proximal and Distal Causation to Gene Loss

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CO-AUTHORS: *Sibei Xu, Troy Sandberg, Elizabeth Brunk, Ying Hefner, Richard Szubin, Adam Feist, Bernhard Palsson*

Biology is characterized by dual causation. The immediate response (i.e., proximal causation) to genetic perturbation is studied by measuring an organism's phenotypic response to a gene knockout (KO). The adaptive response (i.e., distal causation) is studied by measuring changes in an organism's physiology that are required to overcome gene loss through evolution. The latter has been poorly characterized. In this study, using Escherichia coli as a model, a novel experimental design, analytics, and bioinformatics revealed mechanisms and principles of how biological systems respond and adapt to gene KO. First, the proximal response to gene KO was sub-optimal, requiring adaptive evolution to re-optimize function, during which the majority of measured cellular components returned to levels of the reference strain. Second, the molecular phenotype of 24 parallel-evolved endpoints had unique and quantifiable differences that were attributable to acquired mutations. Third, re-optimization during adaptation reflected the coordinated interaction of many layers of cellular function: the initial drivers behind these changes were metabolites that proximally affected gene expression, which were then distally adjusted through mutation. KO-specific case studies presented suggest that dual causation assessment gives deep insights into the role of a gene product in organism function and survival.

O-178	Intergenerational changes in faecal microbiome and metabolome in a C57BL/6J mouse colony

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CO-AUTHORS: *Jocelyn Choo, Paul Trim, Lex Leong, Guy Abell, Carly Brune, Nicole Jeffries, Steve Wesselingh, Neil Dear, Geraint Rogers*

The gut microbiome plays an important role in health and wellbeing. It affects the immune system, metabolism, etc. It is therefore important to define its variability in a widely used animal model, the mouse. The follow-on question is: if there are variations, do these affect the associated faecal metabolome? We investigated two different scenarios. Firstly, a comparison of the faecal microbiome and metabolome of the same strain of inbred mouse populations (C57BL/6J) that were housed in two different animal facilities with different husbandry conditions, including housing and diet. This mimics the typical situation faced by scientists replicating an experiment from the literature. We observed significant metabolomic and microbiomic differences between the two colonies. Secondly, the gut microbiota is strongly influenced maternally through vertical transmission, and the progressive loss or gain of taxa due to alterations in environmental factors could lead to gut microbiota divergence in subsequent generations. This potential generational change was studied by looking at how the microbiome and metabolome of a C57BL/6J mouse colony changed over six generations under identical husbandry conditions. In this study marked divergence in both metabolome and microbiome between the first (founder) generation and the second generation was observed. Interestingly, this shift, though attenuated, continued over the full six generations of this experiment. It is important to note that the magnitude of changes in the faecal metabolome closely follow the gut microbiota variations, therefore indicating potential metabolic consequences that should be investigated following gut microbiota alterations.

O-282	What is the relationship between intracellular and extracellular metabolites?

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Microbial cells secrete many metabolites during growth, which are important intermediates of the central carbon metabolism. This fact has not been sufficiently taken into account by researchers when modelling the metabolism of microorganisms for metabolic engineering and systems biology studies. A lot is known about how microorganisms uptake metabolites, but our knowledge on how and why they secrete different intracellular metabolites is poor. The secretion of metabolites by microbial cells has traditionally been regarded as a consequence of intracellular metabolic overflow. However, we provide evidence based on time-series metabolomic experiments that microbial cells eliminate some metabolites in response to an environmental cue independent of metabolic overflow. Moreover, we provide insight on how this knowledge can benefit metabolic modelling and engineering.

O-304	Combining text mining and metabolic network algorithms to complement and interpret metabolic profiles

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CO-AUTHORS: *Clément Frainay, Sandrine Aros, Nicolas Weiss, Benoit Colsch, Frédéric Sedel, Dominique Thabut, Christophe Junot*

List of discriminating identified metabolites (according to the studied disease between two groups), known as "metabolic profiles", are the observable outcomes of metabolic modulations. Those lists of identified compounds are of great value to better understand the underlying biochemical shifts induced during a disease. Nevertheless, those lists are incomplete mainly due to the nature of LC/MS and the ability to identify compounds. Moreover, the analysis of human biofluids only represents the modulations of metabolites exchanged between the tissue and its environment, overshadowing potential metabolites of interest which are involved in intracellular metabolic processes and not released nor uptaken by the tissue/cell. We propose an approach combining metabolic networks and medical text mining to propose metabolites which may expand the biological interpretation by "filling the gaps" of metabolic profiles. The network strategy is inspired from social network recommendation engines such as the ones used by twitter or media broadcasters. It allows finding upstream and downstream metabolites biochemically related to the ones in the profile. The text mining approach consists in automatically mining the literature to retrieve metabolites that are significantly associated with the perturbation under study. The approach had been successfully applied to high resolution LC/MS metabolomics data obtained on Cerebrospinal fluid (CSF) of patients affected by hepatic encephalopathy. The proposed methodology combined with interactions with analysts allowed increasing the metabolic profile size by 40%. Some of the metabolites suggested by our recommendation system were confirmed using standards. Other propositions were confirmed as metabolites of interests when analyzing patient clinical data.

O-315	A Systems Biology Approach to the Understanding of Asthma Severity through the Integration of Metabolomic, Transcriptomic and Epigenetic networks

PRESENTING AUTHOR: *Rachel Kelly, Brigham and Women's Hospital Harvard Medical School, United States*

CO-AUTHORS: *Bo Chawes, Yamini Virkud, Kevin Blighe, Juan Celedon, Scott Weiss, Jessica Lasky-Su*

Background: Asthma emerges from a complex interplay between genetics and environmental exposures; consequently integrative omics analysis may provide important insights into underlying biology. Methods: 328 children with asthma from the 'Genetic Epidemiology of Asthma in Costa Rica' study underwent metabolomic, transcriptomic and epigenetic profiling in blood. Weighted gene co-expression network analysis (WGCNA) in each dataset was used to independently identify modules of co-regulated metabolites, gene-probes and gene-associated cpg-sites. Modules significantly associated with metrics of asthma severity were identified and their biology explored. Significantly correlated metabolite-gene and gene-gene module pairs were identified and the constituent features submitted for integrated pathway analysis using IMPaLA. Results: WGCNA identified eight metabolomic modules, eight gene-probe transcriptomic modules and ten cpg-site epigenetic modules. Six metabolomic, four transcriptomic and three epigenetic modules associated (p<0.05) with asthma severity based on eigenvalue. The modules were enriched for asthma relevant processes, and between -omic module associations were identified. In particular a 'sphingolipid metabolism' metabolite module associated with a transcriptomic (p=0.05) and epigenetic (p=0.01) module both of which were enriched for immune processes and included ORMDL3; a sphingolipid biosynthesis regulator and validated asthma gene. Conclusions: This study demonstrates that integrating multiple omic technologies in a systems biology approach provides a more informative and biologically meaningful picture of asthma severity. Integration of correlated omic modules expanded and refined the single omic findings, linking dysregulated immunity to asthma severity via ORMDL3 and sphingolipid metabolism. Therefore metabolomics can provide a mechanistic basis for the role of a number of asthma genes.

O-328	Dynamic flux modelling of adipocytes stimulated by insulin using 13C-labelled metabolites

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Humans are exceptional at storing and expending calories, and central to this switch is adipocytes responding appropriately to insulin. 3T3-L1 adipocytes were fed with 13C-labelled glucose, and six metabolites samples were taken across the hour as they transition from basal to insulin-stimulated state. We used B-splines and flux modelling to transform the time profiles of central carbon metabolite abundances and 13C enrichments into flux-concentration models. The models were then interrogated by yield and kinetic analyses to extract key metabolic events and underlying allosteric interactions. Upon insulin stimulation, adipocytes increased conversion of glucose to lactate in an overflow manner, as well as increased flux through the non-oxidative pentose-phosphate pathway and pyruvate carboxylase. The latter demonstrated that insulin increased the reliance on malic enzymes to generate NADPH. Overall, this framework leverages the use of metabolic networks to decipher non-intuitive but highly correlated metabolite abundance and 13C enrichment data. This work contributes toward characterising metabolic signatures of insulin resistance in adipocytes, using the metabolome as an integrative readout of insulin-dependent acute metabolic responses.

O-333	A novel systemic approach to patient stratification

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CO-AUTHORS: *Enrico Glaab, Ronan Fleming, Ines Thiele*

Human diseases involve alternations of the metabolic network. Hence, there is great interest in the identification of metabolites as prodromal, diagnostic, or progression biomarkers. However, the search for these markers proofs to be difficult, potentially because of the clinical heterogeneity among patients. As part of the National centre for Excellence in Research on Parkinson's Disease (NCER-PD) we aim to identify novel biomarker signatures for Parkinson's disease. Clinical heterogeneity in onset, progression, and disease pathology among genetic and idiopathic Parkinson's disease patients suggests the existence of disease subtypes, who could benefit from more targeted disease management. Within the NCER-PD project, we apply a novel systemic approach to stratify patients and computationally predict markers for the disease. We take into consideration targeted serum metabolomics of patients and controls, the network structure of the human metabolic model, and machine learning techniques. The context of the metabolic model, which is based on biological literature knowledge, can highlight functional relationships between altered metabolite levels that could not have been identified from the data alone. We investigate the emerging functional relationships to gain insights into the mechanistic basis of altered metabolite levels and to identify markers to improve the diagnosis of Parkinson's disease. Taken together, we present a systems approach for the analysis of metabolomic data.

O-347	An Integrative omics approach to disentangling the relationship between prenatal vitamin D exposure, 17q21 genetic variants, sphingolipid metabolism, and the development of asthma.

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CO-AUTHORS: *Rachel Kelly, Bo Chawes, Hans Bisgaard, Augusto Litonjua, Yamini Virkud, Scott Weiss*

Background: Prenatal vitamin D supplementation holds the promise for primary prevention of childhood asthma, but may have markedly different preventive efficacy dependent on individual genetic make-up and the primary metabolic pathways through which it acts is unknown. Objective: We utilize metabolomics to disentangle the relationship between two asthma risk factors: 1) prenatal vitamin D supplementation and 2)asthma risk variant(17q21). Methods: Children were genotyped for the functional 17q21 SNP rs12936231 in the VDAART(N=806) and COPSAC2010(N=581) prenatal vitamin D trials. A total of 245 plasma VDAART samples were used to generate metabolomics data. We 1) evaluated the effect of vitamin D on rs12936231 in relation to asthma; 2) identified common metabolites and pathways between vitamin D and asthma; 3) determind what metabolites were causally associated with vitamin D, asthma, and rs12936231; and 4) replicated these findings using an independent sample. Results: We identified an increasing protective effect of vitamin D by decreasing number of 17q21 risk alleles(p=0.066). We subsequently identified 11 metabolites associated with vitamin D and asthma(p<0.05), where sphingomyelin was identified as a significant mediator of the vitamin D induced reduced risk of asthma(p=0.04) and replicated this finding(p<0.05). We then determined that sphingomyelin peak intensity was inversly correlated to the number of 17q21 risk alleles. Conclusions:This study shows that prenatal vitamin D supplementation reduces the risk of childhood asthma through alterations of the sphingolipid metabolism dependent on 17q21 genetic variants, which provides novel mechanistic insight into the pathogenesis of this common childhood disorder and may pave the path for precision prevention.

O-387	Enhancing tetanus toxin production through fermentation maps

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Bacteria produce some of the most potent biomolecules known, of which many cause serious diseases such as tetanus. For prevention, billions of people and countless animals are immunised with the highly effective vaccine, industrially produced by large-scale fermentation. However, toxin production is often hampered by low yields and batch-to-batch variability. Improved productivity has been constrained by a lack of understanding of the molecular mechanisms controlling toxin production. Here we have developed a reproducible experimental framework for screening phenotypic determinants in Clostridium tetani under a process that mimics an industrial setting. We show that amino acid depletion induces production of the tetanus toxin. Using time-course transcriptomics and extracellular metabolomics to generate a 'fermentation atlas' that ascribe growth behaviour, nutrient consumption and gene expression to the fermentation phases, we found a subset of preferred amino acids. Exponential growth is characterised by the consumption of those amino acids followed by a slower exponential growth phase where peptides are consumed, and toxin is produced. The results aim at assisting in fermentation medium design towards the improvement of vaccine production yields and reproducibility. In conclusion, our work not only provides deep fermentation dynamics but represents the foundation for bioprocess design based on C. tetani physiological behaviour under industrial settings.

O-388	Integrative Omics Approach Reveals Coordinate Regulation of Metabolites Glycosylation and Stress Hormones Biosynthesis by TT8 in Arabidopsis

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CO-AUTHORS: *Amit Rai, Megha Rai, Boon Kiat Lim, Johanan Aow Shao Bing, Sanjay Swarup*

Plants defend themselves against various stresses by coordinating response through phytohormones and defense-related secondary metabolites. Glycosylation is one of the major processes that generates structurally and functionally diverse metabolites associated with stress response. Glycosylation involves conjugation of core structures with chemical moieties, such as sugars. While the enzymes involved in glycosylation are known, there are large gaps in understanding the regulation of metabolites glycosylation and its coordination with defense response. To identify gene and metabolite components of the glycosylation machinery, we integrated genomic relationships and gene expression outcomes with metabolomics to characterize TRANSPARENT TESTA 8 (TT8), a transcription factor previously shown to affect flavonoid glycosides. Metabolomics analysis of TT8 loss-of-function and inducible overexpression lines showed that TT8 coordinates glycosylation of nucleotides in addition to flavonoids, thus revealing its key role in regulating activated sugars. Both transcriptome and promoter network analysis revealed that TT8 regulome includes sugar transporters, sugar binding and sequestration proteins, carbohydrate active enzymes and stress response genes. We show that TT8 directly binds to the promoters of key genes in brassinosteroid and jasmonic acid biosynthesis, thus regulating the hormone levels. This combined effect on metabolites glycosylation and stress hormones by TT8 induced overexpression improved germination rates of plants under multiple abiotic and biotic stresses by nearly 30%. Conversely, loss of TT8 lead to increased sensitivity to these stresses. This study provides direct evidence to show that two strategies in plant defense, namely, generation of metabolite diversity and hormone-mediated reprogramming of stress pathways are coordinately regulated by TT8.

O-395	Towards dynamic, genome-scale modeling that captures metabolite levels and regulation

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CO-AUTHORS: *Robert Dromms*

In theory, metabolomics data should be ideal for use in driving genome-scale metabolic modeling, yet to date it is underutilized. Many of the existing modeling tools make assumptions (e.g., that metabolite levels remain at steady state) that preclude direct integration of metabolomics data into the underlying models and that are often not even reasonable. Nonetheless, some of these assumptions and approaches yield attractive computational properties. Retaining these properties while allowing integration of metabolomics data could allow us to drastically improve the predictions of the many analysis tools that use genome-scale metabolic modeling. We have designed, implemented, and characterized a modeling strategy based on a truly dynamic flux balance analysis (DFBA) approach to achieve these goals. This modeling strategy adds constraints describing the dynamics and regulation of metabolism, while (unlike existing approaches) retaining the linear programming structure of FBA. We have evaluated the strategy using a simplified small-scale model to characterize some basic features of its performance, and with a model of E. coli central carbon metabolism to demonstrate the strategy in a physiologically relevant system. We found that our modeling strategy is more robust to having higher noise and fewer samples (exactly the kind of data one would expect from metabolomics experiments) compared to models based on differential equations. The larger E. coli model presented challenges for fitting model parameters; we developed several methods to address this scale-up problem. Our approach represents a promising advance for applications from metabolic engineering to comparative evolutionary analysis.

O-458	Deciphering the metabolic responses to polymyxin killing in Acinetobacter baumannii ATCC 19606 by developing a high-quality genome-scale metabolic model

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CO-AUTHORS: *Jinxin Zhao, Tony Velkov, Jiangning Song, Falk Schreiber, Jian Li*

Acinetobacter baumannii has been recently ranked as No. 1 on the List of Antibiotic-resistant Priority Pathogens by the World Health Organization (WHO). Polymyxins have revived as the last-line therapy against multidrug-resistant A. baumannii. Alarmingly, an increasing number of outbreaks have been reported due to the emergence of polymyxin resistance, which urges the systematic investigation of the mechanisms of polymyxin killing and resistance at network level. To address this, we developed a high-quality genome-scale metabolic model (GSMM), designated as iATCC19606, for A. baumannii ATCC 19606 based on the literature and genome annotation. Flux balance analysis was employed to predict the growth and metabolic phenotypes with iATCC19606 under various nutrient conditions. In silico single-gene deletion was performed to predict gene essentiality. The final model contains 773 genes, 1,190 reactions and 1,131 metabolites, representing the most comprehensive reconstruction for A. baumannii to date. As a result, iATCC19606 well predicted the utilisation of 195 carbon and 95 nitrogen sources. Gene essentiality analysis showed 72.3% accuracy compared with the single-gene deletion library. Integrative analysis with our recent transcriptomics and metabolomics data revealed that polymyxin treatment resulted in significant perturbations in central metabolism, as well as biosynthesis of amino acids, nucleotides and glycerophospholipids. Overall, this is the first integrative study by combining GSMM with multi-omics data to elucidate the metabolic responses to polymyxins in A. baumannii. The model iATCC19606 will be employed as a powerful tool for in-depth investigation of antimicrobial killing and resistance, and the rational design of effective antimicrobial therapies against this problematic pathogen.

O-499	Model-based Engineering of Metabolism

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CO-AUTHORS:

Improved understanding of the organization of metabolic networks can enable the more effective control of metabolism for several applications ranging from metabolite overproduction to treatment of metabolic diseases. Advances in computational modeling techniques have allowed the development of genome-scale models of metabolism in several organisms. These models have become the basis for analysing the potential of metabolic networks and to understand their organization. Using genome-scale metabolic models, we analyze the role of redundancy of metabolite production pathways and its implications for the robust production of the target metabolites. These observations shed light on the role of redundant modes of regulation and metabolic pathways for robust control of metabolic fluxes. In the second part, we will discuss how orthogonality of production pathways can facilitate the effective control of fluxes through target metabolites and their implications for the evolution of modular pathways in metabolic networks. The talk will highlight how these genome-scale metabolic models can be effectivley used for metabolic engineering. Finally, we will conclude with a discussion of the challenges and opportunities in integrating these metabolic models with large-scale metabolomics data.

O-518	Environmental metabolomics with data science as an indicator of ecosystem homeostasis

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CO-AUTHORS:

A natural ecosystem can be viewed as the interconnections between complex metabolic reactions and environments. Humans, a part of these ecosystems, and their activities strongly affect the environments. To account for human effects within ecosystems, understanding what benefits humans receive by facilitating the maintenance of environmental homeostasis is important. Here we describe recent applications of several NMR approaches to the evaluation of environmental homeostasis by metabolic profiling and data science. The basic NMR strategy used to evaluate homeostasis using big data collection is similar to that used in human health studies. Sophisticated metabolomic approaches (metabolic profiling) are widely reported in the literature. Further challenges include the analysis of complex macromolecular structures, and of the compositions and interactions of plant biomass, soil humic substances, and aqueous particulate organic matter. To support the study of these topics, we also discuss sample preparation techniques and solid-state NMR approaches. Because solution and solid-state NMR can produce numerical matrix data (e.g., chemical shifts versus intensity) with high reproducibility and inter-institution convertibility, further analysis of such data using multivariate analysis and machine learning approaches is often desirable. We also describe techniques for data pretreatment in solid-state NMR, for environmental feature extraction from heterogeneously-measured spectroscopic data, and for the extraction of submerged information using machine learning approaches.

P-94	Systems biology of circadian clock synchronization in Neurospora crassa.

PRESENTING AUTHOR: *Michael Judge, University of Georgia, United States*

CO-AUTHORS: *Ricardo Borges, Brooke Hull, Yinwen Zhang, Yueze Yang, James Griffith, Arthur Edison, Jonathan Arnold*

The circadian clock is a widely conserved emergent property driving daily rhythms across multiple levels of biological organization, with oscillations emanating from core transcription-translation feedback loops which then exert control over downstream pathways. In turn, multiple external factors may influence the clock, allowing for re-synchronization with external cues, or compensation for changes in temperature and nutrient availability. We have recently found that transcriptional clock/oscillator phases in single cells of the clock model Neurospora crassa synchronize over time, but only if they are in the same microenvironment. We hypothesize an extracellular chemical communication mechanism to explain synchronization. To explore the nature of this mechanism and the underlying gene-metabolite network, we are using a combination of untargeted NMR metabolomics, clock activity-guided fractionation, and genetic knockout mutants to screen for extracellular molecules and genetic components that may allow clocks to interact. Identifying pathways at the intersection of these approaches will further enable targeted MS experiments and transcript analyses. Preliminary NMR data on nonpolar exometabolites has already revealed circadian features, suggesting interactions of this sort occur in Neurospora. This experiment is being repeated with more replicates, clock mutants, and broader chemical diversity. Our approach will also allow us to explore broader properties of the N. crassa extracellular metabolome in the future. Moreover, this work offers fundamental insights about the control and multilevel properties of a pervasive systems behavior in a model organism with biotechnological relevance.

P-95	Mammalian systems biotechnology reveals global cellular adaptations in a recombinant CHO cell line

PRESENTING AUTHOR: *Hock Chuan Yeo, Bioprocessing Technology Institute, Singapore*

CO-AUTHORS: *Meiyappan Lakshmanan*

Effective development of host cells for therapeutic protein production is largely hampered by the poor characterization of cellular transfection. Here, we employed a multi-omics based systems biotechnology approach to characterize the genotypic and phenotypic differences between a wild-type and recombinant antibody-producing Chinese hamster ovary (CHO) cell line. At the genomic level, we observed extensive rearrangements in specific targeted loci linked to transgene integration sites. Transcriptional re-wiring of DNA damage repair and cellular metabolism in the antibody-producer, via "hard-wired" changes in gene copy numbers was also detected. Subsequent integration of transcriptomic data with a genome-scale metabolic model showed a substantial increase in energy metabolism in the antibody producer. Metabolomics/lipidomics analyses further substantiated this observation, and together with glycomics analysis, highlighted additional characteristics of the antibody producer. These include an elevation in long-chain lipid species, potentially associated with protein transport and secretion requirements, and a surprising stability of N-glycosylation profiles between both cell lines.

P-96	Improved Understanding of the Role of Metals in the Metabolome and Lipidome: A Multi-"omics" Association Approach.

PRESENTING AUTHOR: *Richard Robinson, Metabolon Inc., United States*

CO-AUTHORS: *Matthew Doyle, Anne Evans, Luke Miller*

Metals have long been known to play an important role in human biology with as much as one third of enzymes are thought to utilize metals as catalysts. While the role of some metals in biological systems has been extensively studied the examination of a suite of metals and their interaction across a variety of metabolic pathways has only recently become feasible through instrumentation allowing accurate, high throughput analysis of the lipidome, metabolome and metallome. Here we present an association study, and accompanying methodology, between metals measured by ICP-MS and both small molecules measured by UPLC-MS and lipids measured via FIA-DMS-MS/MS. From a single plasma sample metals (Na, Mg, K, Ca, Sc, V, Cr, Fe, Mn, Co, Ni, Cu, Zn, As, Se, Mo, Ag, Cd) measured via ICP-MS can be compared to small molecule metabolites measured via established non-targeted UHPLC-MS methods and complex lipids measured via FIA-DMS-MS/MS methods. An examination of the correlation between each of the measured metals and each of the measured metabolites and complex lipids was then performed. This approach provides the tool which allow for novel mapping of trace metals onto established biochemical pathways as well as potential elucidation for the role of these metals in disease.

P-97	**Characterising a core metabolic enzyme responsible for phosphine resistance and fundamental metabolic regulation – from classical biology to systems biology and genome-scale modelling**

PRESENTING AUTHOR: *Horst Schirra, The University of Queensland, Australia*

CO-AUTHORS: *Angelo Chan, Jake Hattwell, Paul Ebert*

Phosphine gas is used to protect global grain reserves from pest insects, which are increasingly resistant. We identified dihydrolipoamide dehydrogenase (DLDH) as the enzyme responsible for phosphine resistance and characterised in C. elegans the toxic action of phosphine and the resistance mechanisms with NMR-based metabolomics [1]. DLDH is a core metabolic enzyme, central to metabolic regulation, and a new class of resistance factor. DLDH participates in four key steps of core metabolism, which are affected differently by phosphine in mutant and wild-type animals. The position of DLDH in the metabolic network makes it a highly likely candidate for a central regulator of metabolism. We are studying the role of DLDH in biological/clinical processes, such as lifespan determination, obesity, Alzheimer's Disease, and respiration. Metabolomic analysis indicates a role of DLDH in the cross-talk between branched-chain amino acid and lipid metabolism. We have developed CeCon, a genome-scale metabolic model of C. elegans metabolism that enables further characterization of DLDH's role in these processes. CeCon comprises 225 pathways, 1923 reactions and 1394 metabolites. It also identifies 20793 polypeptides, including 2754 enzymes and 73 transporters. The model forms the basis for creating a C. elegans consensus genome-scale model. DLDH is an exceptional case in which a combination of systems biology methods has identified a single genetic cause of phenotypic change that can subsequently be studied with a wide range of methods from classical biochemistry to systems biology. [1] Schlipalius et al., Science, 2012, 338:807.

P-98	**The ryegrass (Lolium perenne) metabolome: Harnessing chemical diversity and understanding biological mechanisms for forage improvement**

PRESENTING AUTHOR: *Arvind Subbaraj, AgResearch Ltd., New Zealand*

CO-AUTHORS: *Karl Fraser*

We present the ryegrass (Lolium perenne) metabolome, the widest coverage of a LC/GC-MS analysis of oligosaccharides, fatty acid methyl esters (FAMEs), polar, semi-polar and lipophilic/non-polar metabolites, from five clonal replicates of 724 genotypes (3620 plants), representing 118 populations from 21 countries. Perennial ryegrass is the most important forage crop which supports milk and meat production worldwide, and has genetic synteny with other important food crops (rice, wheat and sorghum). An assessment of chemical diversity and understanding biological mechanisms is therefore essential for breeding exercises aimed at forage improvement. Due to its potential impact on ruminant digestion, high water soluble carbohydrate (WSC) content has been a longstanding breeding objective. Chemical diversity based on total WSC content was evaluated, and genotypes were classified as high or low sugar groups. This classification has immediate benefits in directing breeding efforts. Metabolites that were differentially regulated between the two groups were tentatively identified and mapped on to reference pathways. Carbon fixation, carbohydrate metabolism, amino acid metabolism and biosynthesis of secondary metabolites comprised the major networks that were enriched. The shikimate pathway was central to this regulation. Fatty acids, galactolipids, phospholipids and diglycerides were at higher levels in the low sugar group. This also comprises the first report of the ryegrass lipidome. Data-driven hypotheses generated by such large-scale metabolomics studies demand rigorous quality control parameters, and extensive statistical/chemometric evaluations. We present a template for large-scale, non-targeted metabolomics studies along with potential pitfalls and emerging scenarios. A time-resolved analysis of the ryegrass metabolome has also been conducted.

P-99	**Integration of NMR metabolomics, microarray analysis and transgenesis in Anopheles gambiae**

PRESENTING AUTHOR: *Rudi Grosman, University of Liverpool, United Kingdom*

CO-AUTHORS: *Mark Paine, Gareth Lycett, Lu-Yun Lian*

Anopheles gambiae complex include the most important vector of malaria in sub-Saharan Africa and play host to Plasmodium falciparum responsible for the most lethal malaria cases. Alongside treating and curing malaria, prevention plays a critical role in reducing the number of malaria cases. Insecticides are crucial in keeping An. gambiae population under control, however overuse of the insecticides has caused an increase in resistance. Recently, cuticular hydrocarbons (CHC) and metabolic processes have become a point of interest to study insecticide resistance and combating malaria. CHC's have a range of roles including as contact pheromones and waterproof coating, the latter being critical for the survival of pupal and adult stages. Although there are different pathways that produce CHC, a common step in its synthesis is the decarbonylation process carried out by the cytochrome P450 enzymes (CYP-P450). Two CYP-P450s (CYP4G16 and CYP4G17) have been implicated in CHC decarbonylation through previous Drosophila studies, microarray analyses and recombinant protein expression. To observe the metabolic changes and their relation to the CHC pathways, metabolic profiles of transgenic knockdown of the two CYP-P450 enzymes in pupae were studied by nuclear magnetic resonance metabolomics. Multivariate statistical analysis showed significant differences in metabolite levels between pupa knockdowns and control. Metabolites contributing to the separation between groups have been identified as part of the CHC pathway or associated pathways. Of the two knockdowns, metabolite variance of KD16 is more pronounced. This result may be associated with its activity earlier in the life cycle of An. gambiae.

P-100	Establishment of Normalization Protocol for Global Metabolomics in a Large-scale Study Using Mass Spectrometry

PRESENTING AUTHOR: *Daisuke Saigusa, Tohoku University, Japan*

CO-AUTHORS: *Ikuko Motoike, Seizo Koshiba*

Genome, transcriptome, proteome and metabolome are the elements of constituting central dogma, and understanding of the phenotype of an organism. Metabolomics is a promising approach in the search for disease biomarkers because the metabolite concentrations of body fluids are considered as quantitative traits that can describe and define phenotypic characteristics of each individual, which are generated through interactions between genes and environmental influences. Although the quality of metabolome, both global metabolomics (G-Met) and targeted metabolomics using mass spectrometry (MS), largely depends on the instrumentation, potential bottlenecks still exist at data normalization, especially for a large-scale study. Therefore, we established a normalization method of G-Met protocol of data analysis to compensate for intra- and inter-batch differences. In our protocol, samples were deproteinized in a 96-well plate using an automated liquid-handling system, and conducted either using an UHPLC-QTOF/MS equipped with a reverse-phase column (Acquity HSS T3; Waters) or a LC-FTMS equipped with a HILIC column (ZIC-pHILIC; Sequant). Then we applied our protocol for 1,008 plasma samples, which were obtained from our cohort study "Tohoku Medical Megabank Project". All data were imported "Progenesis QI" and normalized by our software "Quantbolome". The variations of 19 plates were significantly reduced using our normalization method. Finally, we demonstrated the association of SNPs by means of genome wide association study between genomics and metabolomics, and identified novel relations in several metabolism. The protocol and normalization method should prove useful for the discovery and development of biomarkers for diseases in a large-scale study.

P-101	Examining the Correlations between Metabolites and Beef Cattle Carcass Data

PRESENTING AUTHOR: *Samantha Connolly, University of Sydney, Australia*

CO-AUTHORS: *Luciano Gonzales, Anthony Dona, Michael D'Occhio, Lorna Wilkinson-White, Darren Hamblin*

Desirable carcass traits are difficult to predict in beef cattle before the animal is slaughtered. The ability to identify animals with superior carcass traits via metabolomics analysis could increase production efficiencies significantly. This is particularly relevant for animals kept in feedlot for prolonged period of time due to its cost. The objective of this study was to search for biomarkers that predict specific carcass attributes in feedlot cattle. Plasma samples have been obtained from a large cohort of animals. These samples have been examined on a Bruker AV 600 MHz NMR spectrometer, Nuclear Overhauser Effect Spectroscopy (NOESY) data was collected for all samples, and phased, baseline corrected and referenced using Matlab™. Chenomx™ and previous publications were then used to classify the metabolites within each individual sample. The metabolites identified were then contrasted with the phenotypic carcass data collected post-slaughter. The correlations between individual metabolites and specific carcass attributes were examined and a search for biomarkers of carcass attributes in plasma samples was performed. The expected outcome of the analysis is to identify a specific metabolite or group of metabolites that are able to routinely predict which animals will have superior carcass attributes.

P-102	Development of a metabolomics method for measuring oxygen-sensitive THF metabolites

PRESENTING AUTHOR: *Renato de Souza Pinto Lemgruber, University of Queensland, Australia*

CO-AUTHORS: *Kaspar Valgepea, Mark Hodson, Ryan Tappel, Michael Koepke, Lars Nielsen, Esteban Marcellin*

Tetrahydrofolate (THF) metabolites (Methenyl-THF; Methylene-THF; Methyl-THF) are the spinal cord of autotrophic growth by the Wood-Ljungdahl pathway found in acetogens (1, 2). Despite their importance, no method is available to measure intracellular concentrations of THF intermediates; most likely because of their rapid degradation in the presence of oxygen (1). Hence, we developed a method for sampling anaerobic cultures to identify and quantify THF metabolites. Culture were injected into an anoxic serum bottle containing anoxic acetonitrile. The samples were then filtered inside the anaerobic chamber and concentrated prior to LC-MS analysis. LC-MS analysis was performed using a Dionex Ultimate 3000 coupled to a QTRAP operated in positive ion mode. Chromatographic separation was achieved using a Phenomenex Gemini-NX C18 column. Comparison of oxic versus anoxic sampling revealed that THFs can only be measured if sampled anoxically. THFs quantification also showed a similar decreasing trend at high biomass, a trend shared by acetyl-CoA, the end product of the WL pathway Although quantification of THFs is likely only reliable using labelled standards, the method developed here is useful for relative quantification of THFs levels across different growth conditions. References: 1. Ragsdale SW. 1991. Enzymology of the acetyl-CoA pathway of CO2 fixation. Crit Rev Biochem Mol Biol 26:261–300. 2. Drake HL, Küsel K, Matthies C. 2006. Acetogenic Prokaryotes, p. 354–420. In Dworkin, M, Rosenberg, E, Schleifer, KH, Stackebrandt, E (eds.), Prokaryotes (Ecophysiology and Biochemistry)Second. Springer, New York.

P-103	An Optimized Metabolome Sampling Methods for 13C Metabolic Flux Analysis of Komagataeibacter xylinus Using Capillary Electrophoresis-Mass Spectrometry

PRESENTING AUTHOR: *Dong Sik Yang, Samsung Advanced Institute of Technology, South Korea*

CO-AUTHORS: *Tae Yong Kim, Sujin Park, Sunghaeng Lee, Hong Soon Rhee, Jin Kyu Kang, Jin Hwan Park*

Komagataeibacter xylinus is one of the microorganisms synthesizing cellulose with higher mechanical strength and degree of crystallinity than plant-based cellulose. K. xylinus has been genetically modified or cultivated on the better environmental conditions to enhance the bacterial cellulose production due to their wide variety of applications such as paper product, electronics and biomedical devices. However, their low productivity is still bottleneck for industrial applications and the information on metabolic fluxes in K. xylinus during cellulose biosynthesis is minimal due to the drawback of sample preparation in metabolomics. To obtain a more comprehensive and better understanding of the bacterial cellulose production pathways , the optimized metabolome sampling methods for 13C metabolic flux analysis were developed. Major metabolome sampling methods optimized included quenching and metabolite extraction: buffered (acetic acid pH 5.5) 100% methanol quenching solution at -40°C and methanol/water/chloroform (2:0.8:2) extraction at 50°C for 30min. The optimized method provided the better quenching effect and resolution of metabolites involved in cellulose biosynthesis and represented an accurate snapshot of metabolic state in K. xylinus using capillary electrophoresis quadrupole time-of-flight (CE-QTOF) due to the absence of cellulase treatment. The optimized method was applied to 13C metabolic flux analysis measuring the mass isotopomer distributions of intracellular metabolites rather than the proteinogenic amino acids to investigate metabolic flux in K. xylinus during cellulose biosynthesis.

P-104	A 1st generation consensus reconstruction of Caenorhabditis Elegans

PRESENTING AUTHOR: *Jake Hattwell, Centre for Advanced Imaging, The University of Queensland, Brisbane, Australia, Australia*

CO-AUTHORS: *Angelo Chan, Paul Ebert, Horst Joachim Schirra, Christoph Kaleta*

To characterise phosphine resistance and metabolic regulation in C. elegans our research group has constructed CeCon, a C. elegans genome scale model (GSM). CeCon comprises 225 pathways, 1923 reactions and 1394 metabolites. It also identifies 20793 polypeptides, including 2754 enzymes and 73 transporters. The model is independent of two other C. elegans GSMs but complements them. We are now working on combining one of these models, ElegCyc, with CeCon to create a consensus GSM containing information from both models. Both CeCon and ElegCyc were constructed in the Pathway Tools software, facilitating easier combination. We have been using the COMMGEN software to merge the two models in a semiautomatic fashion, followed by the use of COBRA techniques to verify that flux is carried. Originally, we compared the two models in several metrics, before exporting both models to SBML for merging. We generated a draft consensus model for C. elegans which can carry flux. Currently we are working on experimentally verifying the draft consensus model by studying the response to dietary perturbation in wild type and mutant worms. In the future, we want to use this model to explore the role of dihydrolipoamide dehydrogenase (DLD) in energy metabolism. We predict that DLD is a key metabolic regulator, as it is shown to have roles in phosphine resistance, lifespan extension, obesity, diabetes, cancer and Alzheimer's Disease. Finally, through the creation of this model we hope to elucidate how DLD mutations affect metabolic function whilst creating a powerful resource for community use.

P-105	GC-MS based metabolomics provides a new approach to elucidate the modes-of-action of fungicides

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CO-AUTHORS: *Zhihong Hu, xili Liu*

The common existence of fungicides resistance in plant pathogen requires new compounds created with different mode of action which make it an urgent need to set up a fast method to discriminate their MOA from the others. In this paper GC-MS based metabolomics was applied to provide an approach to elucidate MOA of fungicides. A sensitive strain of Botrytis cinerea was exposed to EC50 concentration of 16 fungicides with different MOA (?-tubulin, respiratory chain, succinate dehydrogenase, the uncoupler, methionine synthesis, signal transduction, sterol biosynthesis, and Multi site). The mycelia extracts from methyl alcohol and water were analyzed for their "metabolome and metabolic fingerprint" by using gas chromatography-mass spectrometry. The obvious di?erences could be found between these MS vectors pro?les of controls and cultures treated with fungicides pro?les, hence, allowing the classi?cation of fungicides according to their MS vectors pro?les. A model based on hierarchical cluster allowed these antifungal compounds to be distinguished and classified according to their modes of action. Metabolic fingerprinting thus represents a rapid, convenient, and information-rich method for classifying the modes of action of antifungal substances. The metabolic fingerprints have changed with different treatments. The biomarkers of modes of action of fungicides also were established by ANOVA analysis for methionine synthesis inhibitors and signal transduction inhibitors. This study provides a comprehensive database of the metabolic perturbations of Botrytis cinerea induced by MOA diverse inhibitors and highlights the utility of metabolomics for defining MOA, which will assist with the development and optimization of new fungicides.

P-283	The uracil metabolism in the Tetanus vaccine fermentation process

PRESENTING AUTHOR: *Nicolas Zaragoza, AIBN, Australia*

CO-AUTHORS: *Cuauhtemoc Licona-Cassani , Camila Orellana, John Power, George Moutafis, Glenn Moonen, Mark Hodson, Lars Nielsen, Esteban Marcellin Saldana*

Clostridium tetani is an anaerobe pathogen that causes Tetanus by producing one of the most potent toxin known, the tetanus neurotoxin (TeNT). Extensive immunisation programs have kept the disease controlled in humans. However, despite more than half a century of industrial fermentation for vaccine production, the physiological behaviour controlling toxin production in bioreactors is not well understood. This makes production inefficient and costly, with batch-to-batch variability and irregular toxin yields. In order to have an insight of the intricate toxin regulation mechanisms, C. tetani was grown in bioreactors using two conditions, yielding different toxin yields. Using intracellular and extracellular metabolomics, we compared the bacteria metabolic responses to these two conditions and we complemented the approach using time course transcriptomics. We focused our analysis on amino acids, vitamins and nucleotides. The data allowed us to create detailed "fermentation maps" describing C. tetani nutrient consumption and byproducts formation, as well as key metabolites that could be regulating the toxin synthesis pathway. Ultimately, the extensive metabolomics dataset will contribute to a better understanding of the Tetanus vaccine fermentation process and will be used for the future design of a chemical defined medium to avoid the inherent variability.

P-289	Engineering the E.coli lipidome

PRESENTING AUTHOR: *Jos Brouwers, Utrecht University, Netherlands*

CO-AUTHORS: *AikeJeucken, J Bernd Helms*

The model organism Escherichia coli is widely used for industrial production of organic building blocks. A limitation in the production of hydrophobic compounds by E.coli is the destabilization of its lipid membranes and subsequent loss of cell integrity. To increase membrane resistance, we investigated the plasticity of the E coli lipidome by manipulation of the genes involved in lipid synthesis. To this end, we constructed strains overexpressing one of these 74 genes and analysed their lipidomes. We found that 60 of these 74 genes were non-essential and we also analysed knock-out strains of these genes. As determined by principal component analysis, most of these mutants display a lipidome that us clearly distinct from the wild type. We find a remarkable plasticity of the E. coli lipidome, where major lipid species may be nearly completely abolished without effect on growth rate or butanol resistance. With pathway analysis we identify and visualise the relative influence of lipid metabolic genes on the lipidome of E. coli. This work provides a powerful tool for the engineering of E. coli membranes, thus contributing to building a sustainable biobased industry.

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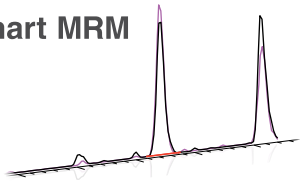

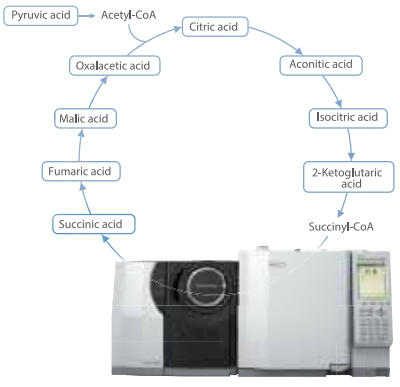

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O-44	MS-based metabolomics reveals BDE47 associated neurometabolic changes

PRESENTING AUTHOR: *JI Fenfen, Hong Kong Baptist University, Hong Kong*

CO-AUTHORS: *Hemi LUAN, Yingyu HUANG, Liangfeng LIU, Min LI, Zongwei CAI*

Polybrominated diphenyl ethers (PBDEs) are one of the major persistence organic pollutants (POPs). As the most abundant congener detected in human serum, BDE47 was studied to have neurodevelopmental toxicity in mice experiment by measuring their spontaneous behaviors. In our study, adult C57BL/6J mice were gavaged daily with BDE47 (0, 1, 10 or 100mg/kg bw) for 30 consecutive days. Every group had eight mice. The collected serum and brain samples were analyzed by LC-MS using an orbitrap fusion mass spectrometer. MS profiling data was preprocessed by using XCMS and the resulting peaks were fitted in partial least squares discriminant analysis (PLS-DA) model. Our result indicated the disturbance of tryptophan and phenylalanine pathways. Significant decrease of phenylalanine, tyrosine, L-DOPA and dopamine (neurotransmitters) were detected by LC-MS/MS quantification. Also metabolites of kynurenine, 3-hydroxykynurenine which was known as a neurotoxin were upregulated in the exposure groups. Based on the results, we suggest the mice receiving BDE47 suffered from considerable neuro-metabolic alteration by disturbing the tryptophan and phenylalanine metabolism. We further evaluated the BDE47 exposure as a potential risk factor of Parkinson's disease (PD) development in the alpha-synuclein overexpressed fly (Drosophila) model. Flies were exposed to BDE47 (2, 10, 50μM) for 30 consecutive days. The regulation of key metabolites include tyrosine, dopamine and 3-hydroxykynurenine in the flies in the 20th exposure day were found to be consistent with the mice experiment. Our results suggest that BDE47 may worse the PD development by disturbing tryptophan and phenylalanine metabolic pathways.

O-69	Rice metabolomics: unravelling brown planthopper (BPH) resistance mechanism on BPH-resistant traits

PRESENTING AUTHOR: *umaporn uawisetwathana, National Center for Genetic Engineering and Biotechnology, Thailand*

CO-AUTHORS: *Olivier Chevallier, Yun Xu, Supavadee Yatapan, Wintai Kamonsukyunyong, Theerayut Toojinda, Apichart Vanavichit, Roy Goodacre, Christopher Elliott, Nitsara Karoonuthaisiri*

Brown planthopper (BPH) is a phloem feeding insect that causes the annual disease outbreak, called hopperburn in many countries throughout Asia. As Thailand is one of the major rice exporters, this outbreak critically reduces the rice production yield, consequently affecting the global rice security. Currently, BPH resistance mechanism is still unknown causing lower progress on developing effective rice varieties as well as effective farming practices. Understanding the biological mechanism of the response to the BPH will aid in improving a selective breeding program of such desirable trait for durable resistance. In this study, leaf extracts of BPH-susceptible Thai Jasmine rice (KD cultivar) and its BPH-resistant rice isogenic lines (IL7 and IL308) were investigated to decipher rice metabolic responses during 8 days of BPH attack. Polar metabolomes obtained by multi-platform metabolomics analyses, namely UPLC-QToF-MS/MS and UPLC-Orbitrap-MS/MS were fused and subsequently analyzed. Multivariate statistical model was capable of distinguishing the metabolic profiles between the BPH-susceptible and BPH-resistant varieties during BPH infestation. Over 100 primary and secondary metabolites were identified and overall metabolic perturbation underlying these traits was highlighted. This study provides a comprehensive insight into biochemical processes of rice adaptation and/or resistance against the BPH infestation. The findings from this study will help scientists narrow down their further investigation of pathways putatively involved in the BPH resistance mechanism of the rice in order to find an effective and sustainable alternative to prevent and control the damage from the infestation.

O-71	An inducible system for anthocyanin accumulation in plants for application in green systems biology

PRESENTING AUTHOR: *Robert Hall, Wageningen Plant Research, Netherlands*

CO-AUTHORS: *Nikolay Outchkourov, Rumyana Karlova, Matthijs Hölscher, Xandra Schrama, ikram Blilou, Esmer Jongedijk, Carmen Díez-Símoin, Aalt-Jan van Dijk, Dirk Bosch, Jules Beekwilder*

Plants regularly respond to multiple (a)biotic environmental stimuli by making secondary metabolites. Regulation of the relevant biosynthetic pathways is part of a complex transcriptional network which ensures that biosynthetic enzymes, transporters and storage capacity are present at the correct time and place. To achieve this, co-regulation of various processes beyond the relevant biosynthetic pathway is also needed. To study such complexity, an 'inducible system' could prove very valuable to follow the coordinated induction of biosynthesis, transport and storage, in a tissue- and development-independent manner. We have developed such a system for studying the program of anthocyanin-biosynthesis in tomato. A transcription factor pair from Antirrhinum was introduced in tomato under control of a dexamethasone-inducible promoter. This system allows the induction of anthocyanin formation within 24h in a variety of tissues. LC-MS based metabolomics in combination with transcriptomics profiling were used with several tomato tissues. The data set obtained provided a comprehensive overview of the processes accompanying anthocyanin biosynthesis in these materials. More than 80 metabolites and 425 genes were found to be regulated by activation of the anthocyanin pathway, independent of the tissue tested. In addition, tissue-specific changes in gene expression and metabolite profiles were also observed. This approach has revealed the broad impact of inducing anthocyanin accumulation in tomato which involves e.g. other unrelated biochemical pathways as well as root branching, epithelial morphology, seed germination and leaf conductance.

O-86	Mapping carbon fate during coral bleaching: the application of 13C metabolomics

PRESENTING AUTHOR: *Katie Hillyer, Victoria University of Wellington, New Zealand, New Zealand*

CO-AUTHORS: *Daniel Dias, Adrian Lutz, Ute Roessner, Simon Davy*

Coral reefs provide critical goods and services, however these systems are globally threatened. A major driver of change is thermal stress associated with rising seawater temperatures, leading to coral bleaching. Where functional, coral reef systems are highly productive, due to an efficient symbiosis between corals and dinoflagellate algae, hosted within their cells. This nutritional relationship is however, sensitive to small temperature changes; when crucial thresholds are exceeded, thermal stress results in the loss of algal symbionts (bleaching). Major gaps still exist in our understanding of change in symbiosis metabolic function during bleaching, however metabolomics is providing important insight into these alterations and the mechanisms of bleaching. We applied a stable isotope tracer (13C bicarbonate) coupled to gas chromatography-mass spectrometry, to map the fate of photosynthetically fixed carbon, during thermal stress and bleaching in both partners (symbiont and host) of a model cnidarian symbiosis (Aiptasia) and a reef-building coral (Acropora aspera). We detected clear metabolic and cellular responses to thermal stress, which progressed with bleaching extent (both partners and symbioses). Primarily, increased energy store catabolism, production of antioxidants and compatible solutes, coupled to reductions in complex biosynthesis pathways. However, despite the metabolic costs of advanced symbiont photodamage, remaining symbionts in both symbioses continued to fix carbon, produce organic products de novo and translocate to their hosts (primarily as glucose). This on-going translocation during bleaching, suggests that cnidarian hosts may manipulate symbiont nutrient provision, or target non-productive symbionts for expulsion, providing important insight into the mechanisms of coral bleaching.

O-135	Geographic Mosaic of Metabolites in Purple Passionflower (Passiflora incarnata): Testing the Coevolutionary Hypothesis Using 1D 1H-NMR

PRESENTING AUTHOR: *Nick Batora, University of Georgia, United States*

CO-AUTHORS: *Ricardo Borges, Charalampos Panagos, Rodney Mauricio, Arthur Edison*

Plants produce an outstanding diversity of metabolites and natural products. Insights into the ecological and evolutionary forces that have helped generate this great diversity is still limited. One hypothesis is that over time coevolutionary dynamics between plants and specialist insect herbivores led to a greater diversity of secondary metabolites. We tested predictions based upon the coevolutionary hypothesis within a species using Purple Passionflower (Passiflora incarnata). We utilized a 1D 1H-NMR based metabolomics approach to assess metabolic diversity among wild populations of P. incarnata growing in a common garden environment. Populations selected for this study differed in the presence and abundance of specialist herbivores in their respective herbivore communities. We found that populations with more specialist herbivores produced a greater diversity of metabolites. Additionally, we found metabolites that are affected by herbivory from the specialist Lepidoptera, Gulf Fritillary (Agraulis vanillae). This work provided novel metabolites to puruse in future studies that investigate plant metabolites under natural selection from insect herbivores. Furthermore, this study demonstrates the tremendous opportunity metabolomics provides in the evolutionary and ecological study of plant metabolites and has applications in natural product discovery.

O-148	Actylcholine regulation after single pesticide exposure in relationship to developmental neurotoxicty, behavior and cognitive studies in mice

PRESENTING AUTHOR: *Pim Leonards, VU University, Netherlands*

CO-AUTHORS: *Henrik Viberg, Iwa Lee, Sonja Buratovic, Per Eriksson*

Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. Various epidemiological studies indicated that exposure to low doses of environmental active contaminants during human development can have deleterious effects on cognitive development in childhood. In the current study, which was part of the EU DENAMIC project, the aim was to investigate the behaviour and cognitive effects of environmental contaminants in mice and to study the underlying molecular mechanisms of the observed effects using metabolomics. Male mice were exposed to a single dose of pesticides (chlorpyrifos, carbaryl, cypermethrin, endosulfan), methylmercury or PFHxS (PND10). The studies showed that all chemicals caused developmental neurotoxic effects, even after a single exposure which was given at a vulnerable period of brain development. The cognitive and behaviour tests showed that the early life exposure of the compounds can alter adult spontaneous behavior and cognitive function. Interesting the chemicals with different modes of action had effects on the same apical endpoint (increased spontaneous behaviour). Metabolomics of brain tissues (cerebral cortex and hippocampus) showed effects related to axons/neurons, mitochondria, purine pathways. After pesticide exposure increased levels of the neurotransmitter acetylcholine in the hippocampus were found, which was not related to the acetylcholinesterase activity. Acetylcholine is probably regulated to a spontaneous transfer of acetyl by acetylcarnitine as both carnitine and choline were not changed. Combining all studies a positive relationship was found between acetylcholine and acetylcarnitine in the hippocampus.

O-149	Metabolomics analysis of community interactions: the effect of species distributions and spatial heterogeneity on metabolic response.

PRESENTING AUTHOR: *Jade O'Leary, Cardiff University, United Kingdom*

CO-AUTHORS: *Dan Eastwood, Carsten Müller, Lynne Boddy*

Species interactions mediate community dynamics, but few experimental studies have assessed the metabolic processes that govern competitive interactions within communities. Wood decay basidiomycete fungi are model organisms for studying community dynamics because they are easily manipulated in laboratory microcosms, and interactions resolve themselves within a few months. A previous study assessed the interactions of a three-species community in novel "Rubik's cube" 3-dimensional systems. The elimination of patch fragmentation gave rise to emergent properties, namely increased competitive ability, which mediated coexistence, however, the mechanisms responsible for this were unclear. The current study, therefore, investigated the metabolic processes involved in the interactions of a community of wood decay fungi in systems where a single species occupied a greater initial patch size (although the same total area as its competitors), compared to when all species were evenly dispersed throughout the system. Volatile organic compounds (VOCs) were sampled from the headspace of interactions and analysed by TD-GC-TOF-MS, and enzyme assays and NMR and LC-MS metabolomics were performed on extracts of those samples. The bouquet of VOCs differed markedly between system types, and the metabolic and enzymatic profiles of individuals changed in response to the increased competitiveness of the species occupying a greater initial patch size, which in the real-world is likely to be reflected by changes to decomposition and nutrient cycling.

O-150	The fate of technical-grade chlordane in mice fed a high-fat diet and its roles as a candidate obesogen

PRESENTING AUTHOR: *wentao zhu, China Agricultural University, China*

CO-AUTHORS: *Dezhen Wang, Yao Wang, Jin Yan*

Epidemiological studies indicate that exposure to persistent organic pollutants is positively associated with the prevalence of obesity. To delineate the potential role of technical-grade chlordane in obesity development, chlordane metabolism and chlordane-induced metabolic changes were investigated in mice fed high-fat diet (HFD) over a 6-week period. Gas chromatography electron capture detector analysis showed that HFD induced more accumulation of technical chlordane in the liver, muscle and adipose tissue. The enantioselectivities of oxychlordane in selected tissues were also influenced by HFD. 1H NMR-based liver metabolome indicated that technical chlordane can enhance the metabolic alterations induced by HFD. Compared with the low-fat diet (LFD) group, no differences were observed in the LFD+chlordane group. However, as many as 16 metabolites were significantly different between the HFD group and HFD+chlordane group. Moreover, compared to the LFD+chlordane group, the abundances of 24 metabolites significantly increased or decreased in the HFD+chlordane group. Twenty metabolites were altered in the HFD group compared to the LFD group. Tryptophan profiling suggested that both chlordane and HFD can disturb tryptophan catabolism. These interactions between technical chlordane and HFD suggest that technical chlordane is a candidate obesogen.

O-161	Challenging in Plant Metabolomics: How can we observe and interpret metabolomic data?

PRESENTING AUTHOR: *Miyako Kusano, University of Tsukuba, Japan*

CO-AUTHORS:

Metabolomics has become a powerful approach that has been widely adopted, especially in the context of biochemical phenotyping of biological systems. As plants cannot move by themselves (they can, but the movement is limited), they produce primary metabolites to obtain energy to keep their life and secondary metabolites to survive under the given environments. It is estimated that approximately 200,000 metabolites are produced in the plant kingdom. However, there is no single technique suitable for measurement of all metabolites because of the chemical diversity of cellular metabolites and their broad dynamic range, particularly as this pertains to plants. We have put our effort to develop plant metabolomic platforms to detect diverse metabolites in not only model plants but also in crops and vegetables. In plant science, metabolomic approaches are increasingly used for obtaining insights involved in genotype comparison, stress responses, nutrition assimilation and evaluation of genetically modified plants. Metabolomics also applied to extract hidden metabolic networks in plants. Data interpretation of metabolite profiles can give novel insights as physiological meaning in plants. Here I would like to share with you our research activities to uncover mechanism(s) to maintain metabolic status under different stress- or nutrition conditions in the model plants, Arabidopsis (Arabidopsis thaliana) and rice (Oryza sativa). As applied science, I would present several examples to evaluate "quality" of plants including crops, vegetables and fruits in terms of metabolite composition.

O-167	An interatomic based approach to study the influence of pyrimidine availability on the switch from free-living to biofilm growth in Salmonella

PRESENTING AUTHOR: *Anna Yssel, KU Leuven, Belgium*

CO-AUTHORS: *Hans Steenackers*

Although we often think of bacteria as free-living single celled organisms, they often live in complex multicellular communities that are enclosed by a self-produced matrix, called biofilms. Biofilms are problematic in the clinical, industrial and food processing environments. It has been estimated that biofilms are associated with 65 percent of nosocomial infections and that treatment of these biofilm-associated infections costs greater than $1 billion annually in the United States alone. The switch from free-living to biofilm associated growth is complex and tightly regulated in response to environmental and internal factors and involves the use of nucleotide derived signaling molecules to facilitate changes on transcriptional, translational and post-translational levels. We have used various state-of-the-art techniques time-lapse studies with GFP-promotor fusions, LC-MS/MS, RNA sequencing and an interatomic approach to determine how pyrimidine availability influences the molecular-decision making systems of Salmonella enterica serovar Typhimurium that control the switch from free-living to biofilm associated growth We found that pyrimidine starvation leads to biofilm inhibition and an accumulation of purine nucleotides, and surprisingly an increase c-di-GMP (a positive regulator of biofilm formation). Using RNA sequencing and a network based approach we were able to identify the regulatory mechanisms that are responsible for repressing curli production (a major component of biofilms) despite high c-di-GMP levels. Our results demonstrate how the availability of important resources such as nucleotides can influence how bacteria respond to environmental cues.

O-271	Can metabolomics approaches be used to determine the impact of pollution in estuarine environments?

PRESENTING AUTHOR: *Sara Long, University of Melbourne, Australia*

CO-AUTHORS: *Georgia Sinclair, Allyson O'Brien, David De Souza, Konstantinos Kouremenos, Saravanan Dayalan, Ary Hoffmann, Malcolm McConville, Dedreia Tull, Michael Keough*

Biomarkers are often used in environmental monitoring and management to assess responses to pollution in resident biota. Biomarkers need to be sensitive to pollution at environmentally relevant concentrations and ideally respond to specific chemical classes so they can be used diagnostically. Current biomarkers tend to focus on single endpoints in specific taxa that theoretically reflect the response of whole communities, but collection and identification of specific taxa often requires taxonomic expertise and a high level of sample effort that is impractical. Metabolomics techniques have the potential to identify sensitive small metabolite biomarkers that respond in the same way across taxonomic groups to chemicals from similar classes (e.g. metals or pesticides). We investigated this idea using two estuarine invertebrates (a worm and a snail) exposed to two priority chemicals detected in estuarine environments (zinc, a heavy metal, and boscalid, a fungicide). Individuals were exposed to a sub-lethal concentration of each chemical for 48 hours. Whole body homogenates were extracted, and then a multi-platform metabolomics approach (GC/MS for polar metabolites and targeted LC/MS for amine-containing metabolites) was used to determine effects on a number of biochemical pathways. Worms were more sensitive than snails to both chemicals. Amino acids and sugars were the main metabolite classes affected by exposure, with different metabolites altered by zinc compared to boscalid. These results demonstrate the potential for metabolomics approaches to be used diagnostically in biomonitoring programs as they have been able to differentiate responses between different chemicals.

O-401	Metabolomics study of triphenyl phosphate in earthworm Perionyx excavatus: biotransformation products and toxicity

PRESENTING AUTHOR: *Lei Wang, National University of Singapore, Singapore*

CO-AUTHORS: *Xulei Huang, Anna Karen Laserna, Sam Li*

Triphenyl phosphate (TPP) is widely used as flame retardant since the phase-out of polybrominated diphenyl ethers, and has been detected in all environmental compartments. So far, little work has been done on its biotransformation and toxicity in terrestrial ecosystem. We investigated the metabolism of TPP and the perturbation of the endogenous metabolome in the earthworm, Perionyx excavatus, after acute exposure to TPP for one and two days, as well as after chronic exposure for 28 days, using liquid chromatography –tandem mass spectrometry and gas chromatography –mass spectrometry. TPP underwent quick hydrolysis and oxidative hydroxylation after entering into earthworm body. A range of novel phase II metabolites were identified, which can be categorized to thiol conjugations and phosphate conjugations. Significant perturbation of the endogenous metabolome was only observed two days after acute exposure with up-regulation of glucose, certain amino acids and fatty acids, and down-regulation of maltose and certain glycerophospholipids. These results suggest that TPP does not accumulate in earthworm and may not be toxic to earthworm in environmentally relevant scenarios.

O-447	**A systems analysis of source-to-sink and metabolic regulatory mechanisms in tomato fruit development by narrowband-LED light treatments**

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CO-AUTHORS: *Makoto Kobayashi, Tomoko Nishizawa, Shoko Hikosaka, Eiji Goto, Kazuki Saito, Miyako Kusano*

Tomato (Solanum lycopersicum) is one of the most important crops. Due to the physiological importance of the storage metabolites (e.g., carbohydrates), understanding metabolism for translocation from source to sink organs is of interest. Here we conducted integrative omics analyses to elucidate metabolic impact caused by red light emitting diodes (LED) (a peak wavelength of 660 nm) treatment to a single leaf with different intensities during fruit development in tomato plants. To this end we designed and set up the special light irradiation system, called "simplified source-sink model", which consists of a single tomato leaf and fruit truss using red LED lighting during development. We evaluated the fruit size under different light intensities, suggesting that tomato fruit was significantly increased more than 500 mmol m-2 s-1 for 2 weeks after anthesis. We investigated transcriptomic and metabolomic changes of leaf and fruit samples by using microarray, RNA sequencing, and gas chromatography-mass spectrometry. These analyses showed the metabolic shifts in carbohydrate metabolism and in several key pathways which contributed in the fruit development. Our findings suggest that the developed workflow provides a promising way to discover key metabolites in central metabolism that contribute to increase fruit size of tomatoes.

O-477	**Comparative metabolomics of xylose-fermenting yeasts by UHPLC-MS/MS: effects of oxygen levels on fermentation performance**

PRESENTING AUTHOR: *Patrícia Abdelnur, Embrapa Agroenergy, Brazil*

CO-AUTHORS: *Christiane Campos, José Antônio Ribeiro, Patrícia Costa, Henrique Veras, Clenilson Rodrigues, Nádia Parachin, João Ricardo Almeida, Patrícia Abdelnur*

Xylose fermentation is a bottleneck in second generation ethanol production since few yeast species are able to convert this sugar to ethanol. Efforts to improve yeast fermentation performance through genetic engineering have been done, however ethanol yield and productivity are still low and industrial conditions. In this work, a metabolomics platform based on Ultra High Liquid Chromatography coupled to tandem Mass Spectrometry (UHPLC-MS/MS) was developed to quantify key metabolites from central carbon metabolism (glycolysis, pentose phosphate pathway, and tricarboxylic acid cycle) in yeast. Afterwards, comparative metabolic flux analysis was performed to identify limiting steps on xylose metabolism. Spathaspora arborariae and S. passalidarum were cultivated in xylose media under aerobic and oxygen-limited conditions to investigate the oxygen influence in the xylose consumption. A total of nineteen metabolites were quantified in each yeast sample. To our knowledge, for the first time, intracellular metabolites from S. arborariae and S. passalidarum were successfully quantified. Growth under aerobic and oxygen limited conditions, lead to respiratory and fermentative metabolism, respectively. In the last case, cofactor regeneration was hindered and resulted in xylitol secretion significantly, especially for S. arborariae. These results indicated that fine control of oxygen levels during fermentation is necessary to optimize ethanol production with naturally xylose-fermenting yeasts. The metabolomics protocol developed in here can also be applied to other species.

P-106	Metabolic profiles-based alterations as an induced response on Lepidoptera-Lupinus interactions

PRESENTING AUTHOR: *Sandra Vargas Medina, Nueva Granada University, Colombia*

CO-AUTHORS: *Ericsson Coy-Barrera*

Plants respond to phytophagous insects through synthesis of structurally diverse defensive compounds to counteract herbivory-derived effects. Secondary metabolites could be subdivided into constitutive or induced and are then considered highly dynamic depending on plant damage level closely related to insect feed type. However, our understanding of plant-insect defense mechanisms is still limited. Metabolomics provides an opportunity to study plant secondary metabolism-mediated responses on plant-phytophagous interactions. A qualitative UFLC-ESI-HRMS-based metabolic fingerprinting method for untargeted analysis of leaves of a native Andean Lupinus plant (L. bogotensis) and feces of two Lepidoptera larvae species (Pyralidae and Noctuidae) was developed. Analyses revealed changes in alkaloid and phenolic plant composition. Directly-affected plant material by phytophagous-exerted mechanical damage showed the lowest ethanolic extraction yield but it globally presented greater abundance and quantity of alkaloids. On the other hand, unaffected leaf samples showed phenolics-enriched profiles. According to principal component analysis (PCA), Pyralidae larvae attack induces spartein and hydroxyisospartein production. Also, Pyralidae larvae were found to be capable to structurally alter the major alkaloid composition (lupanin dehydrogenation and sparteine monooxygenation/dehydrogenation), whereas Noctuidae larvae were able to consume defensive compounds and eliminate them through excretory system without transformation. On both interactions, greater expression of tetrahydroxystilbene after larvae were fed on plants can be associated to an herbivory-induced response according to PCA model (R2Xcum: 0.756 and 0.748, respectively) and Monte-Carlo cross validation (MCCV) (area under ROC curves: 0.957 and 0.924, respectively). Developed methodology is the first evidence of metabolic-mediated behavior against phytophagous insects from an Andean lupine.

P-107	Investigating the Porites compressa Coral Holobiont, a Complex Symbiotic System, to Understand Mechanisms of Growth Anomalies

PRESENTING AUTHOR: *Tracey Schock, National Institute of Standards and Technology, United States*

CO-AUTHORS: *Erik Andersson, Russell Day, Joseph Stewart, Thierry Work*

Thriving coral ecosystems entail a complex community structure with dynamic biochemical intercommunication. The coral holobiont is an intimate symbiotic community comprising a coral host and associated intracellular microorganisms, bacteria and dinoflagellates. To date, coral research in terms of metabolomics, is restricted to a handful of manuscripts with only one study investigating effects from an environmental stressor. Collecting coral samples in a manner that preserves the community structure and the metabolome of interest is difficult and is likely a reason for the limited studies. Here, we developed stringent collection and processing protocol in order to investigate Porites growth anomalies (GA), a lesion characterized by localized increased skeletal growth resulting in an abnormal protuberant mass on a coral colony. Porites GA are widespread in the Indo-pacific and are associated with human population density. Paired fragments comprising lesion (GA) and healthy Porites compressa were collected from Coconut Island (Oahu, Hawaii) where prevalence of growth anomalies is high. Sample handling was done so as to allow assessment of specific layers of the coral structure (tissue, skeleton, and a mixed layer of tissue and skeleton) by a multi-faceted omic approach, initiating with 1H NMR-based metabolomics. Evaluating the metabolome of the disease state in conjunction with complementary ICP-mass spectrometry trace element and isotopic data on the skeletal anomalies will provide a novel and diverse insight into the biochemistry associated with the disease.

P-108	Characterization of metabolic responses of Nicotiana attenuata during infection with Rhizophagus irregularis by non-targeted high resolution LC-QTOF profiling.

PRESENTING AUTHOR: *Terence Scanlon, Bruuker, Australia*

CO-AUTHORS: *Sven Helling, Ming Wang, Aiko Barsch, T Baldwin, Terence Scanlon, Emmanuel Gaquere*

Mycorrhizal associations are ancient and phylogenetically widespread in >80% of land plant species. AMF are known to improve the water uptake and nutritional resources of host plants. In many cases symbiosis with arbuscular mycorrhizae positively influences growth and enhances plant resistance against pathogens and abiotic stresses. However the effects of AMF on plant defense mechanisms has only been investigated in a few studies. For this purpose, we studied untargeted LC-MS/MS metabolic profiles of N. attenuata tissue extracts from plants growing in either autoclaved or non-autoclaved, active AMF inoculums. We identified 126 differentially regulated features and observed that varying levels of AMF infection were associated with changes in the phytometabolome. By investigating known defense compound classes, differences in 17-hydroxygeranyllinalool diterpene glycosides (HGL-DTGs), polyamine-hydroxy-cinnamic acid esters (phenolamides), depsides and alkaloids could be determined. We show that only the malonylated HGL-DTGs were decreased in plants infected with AMF. Using an in-house MS/MS database consisting of approximately 400 putatively identified metabolites, and by performing an MS/MS correlation search, we identified 6 novel phenolamides which are highly increased in plants infected with AMF. These compounds are specifically characterized by losses of a double bond in their caffeoyl or feruloyl moieties. The described combination of ecological screenings and unbiased metabolite profiling allows for the identification of potential new compounds and metabolic pathways important for the interactions between AMF and N. attenuata

P-109	Impacts of high carbon dioxide and nutrients to the metabolic pathway of oxalate rich plant

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CO-AUTHORS: *Maki Kawai-Yamada*

Rumex obtusifolius (Polygonaceae), one of the most invasive weeds in all over the world, accumulates 10-30% dry weight of soluble oxalate in leaves. Excess intake of oxalate-rich plant is injurious for human and livestock due to mineral lacking and urinary syndrome such as kidney stone. On the other hand, oxalate is useful dicarboxylic acid for plants in detoxification of metal ions such as aluminum ion, defense against predators, a precursor of H2O2 in wounding or aging. Thus, oxalate accumulation would give advantages to adapt various environments for R. obtusifolius. For oxalate synthesis, three pathways are reported in plants: isocitrate pathway, glycolate pathway and ascorbate pathway. However, it had not been cleared which pathway contributes to oxalate accumulation in plants, and what kind of factors affect oxalate synthesis. To clear the effects of environmental factors on oxalate synthesis, we analyzed R. obtusifolius L. grown in high CO2 and nutrient solution. Metabolome analysis using CE-MS revealed that the treatment of high CO2 combined with Hoagland's solution increases the plant biomass and oxalate accumulation via the isocitrate pathway. These results suggest that the oxalate-rich plants would explosively produce itself in the nutrient-rich field with elevated CO2 in the future.

P-110	A three-phase extraction method for the reproducible, comprehensive and step-wise analysis of metabolites and lipids, glycogen and proteins from a single sample

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CO-AUTHORS: *Krzysztof Bajdzienko, Ralf Pflanz, Herbert Jäckle, Patrick Giavalisco*

Sample amounts can be often limiting for the comprehensive analysis of animal or human tissue samples. An all-in-one three-phase extraction protocol, based on a single sample aliquot can help to reduce the amount of material needed in such experiment. Many compound specific extraction protocols are covering only limited numbers of metabolite classes, e.g. lipids or sugars. This results in an increase in sample aliquots needed when conducting analysis of multiple compound classes including lipids, polar metabolites and proteins. The all-in-one extraction method presented here overcomes the problem of multiple sample aliquots and allows for the identification and quantification of glycogen, lipids, metabolites and proteins from a small amount of a single sample. I employed and optimized a liquid-liquid extraction method using the animal model organism Drosophila melanogaster. The three-phase MTBE:MeOH:H2O extraction system comprises of an upper lipid-containing (MTBE) phase, a lower polar metabolites-containing (MeOH:H2O) phase and a solid pellet, which contains both, proteins, glycogen and insoluble polymers (in the case of Drosophila the chitin of the exoskeleton). To identify and quantify the individual compounds different analytical methods and instrumentations were employed. In a proof of concept experiment I will provide data from a small sample set using reversed phase liquid chromatographic (LC) mass spectrometer (MS) for the analysis of more than 300 lipid species, next to gas chromatography (GC) MS analysis of primary metabolites. Additionally, proteins and glycogen, from the precipitated pellets, were analyzed using nano-LC-MS and an enzymatic assay, respectively.

P-111	NMR-based metabolic profiling of rice (Oryza sativa L.) grown under a free-air CO2 enrichment (FACE) field experiment.

PRESENTING AUTHOR: *Yasuyo SEKIYAMA, Food Research Institute, NARO, Japan*

CO-AUTHORS: *Toshihiro Hasegawa, Hiroshi Ono, Hidemitsu Sakai, Yasuhiro Usui, Takeshi Tokida, Hirofumi Nakamura, Jun Kikuchi*

Global climate change is projected to have significant impacts on crop production. Previous studies show that elevated atmospheric CO2 concentrations (E-[CO2]) enhance photosynthesis, as well as rice growth and yield, but decrease grain appearance quality. The yield and quality responses differ depending on cultivars, but the underlying mechanism is poorly understood. Metabolite signatures measured with a field metabolomics approach may provide insight into such mechanisms. Here we report the NMR-based metabolic profiling of two cultivars, differing in response to E-[CO2], grown at the Tsukuba Free-Air CO2 Enrichment (FACE) facility (Ibaraki, Japan) to test the effects of E-[CO2] on rice paddy under open-field conditions. A high-yielding indica cultivar, Takanri, and a standard japonica cultivar, Koshihikari, were grown in 2012 at ambient (383 ppm) and elevated CO2 (578 ppm), and the aboveground organs were sampled at panicle initiation (early July), heading (early August) and mid-grain filling (late August). The 1H NMR spectra of metabolites soluble in a D2O-based buffer were measured and subjected to multivariate or univariate analysis. Principal component analysis showed class separation corresponding to the organs, cultivars, and growth stage of the plants. The effect of E-[CO2] on metabolite profile was greater in the leaf blade of Koshihikari at panicle initiation and of Takanari at heading. Details of the metabolic changes will be presented. References Hasegawa et al., Improving Modeling Tools to Assess Climate Change Effects on Crop Response (eds Hatfield JL, Fleisher D) (2015). Nakamura et al., Journal of Agricultural Meteorology (2012). Usui et al., Global Change Biology (2016).

P-112	Using Metabolomics to Assess the Early Effects of Zinc and Boscalid On Estuarine Polychaete.

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CO-AUTHORS: *Allyson O'Brien, Mick Keough, Dedreia Tull, David De Souza, Saravanan Dayalan, Sara Long*

Early detection of chemicals that cause adverse effects on organisms in aquatic ecosystems is crucial to protect diversity and to maintain ecosystem functions. Metabolomic approaches have recently shown promise to develop sensitive small metabolite biomarkers of low chemical exposure. Zinc is a persistent heavy metal commonly detected in most urban estuary and wetland sediments around Australia. Boscalid is a fungicide that has been routinely detected in estuaries (both in water and sediment) as result of agricultural run-off around Victoria. This study was a time-course experiment aimed at determining the metabolite changes in Simplisetia aequisetis (an estuarine polychaete), following exposure to a sub-lethal concentration of both zinc and boscalid in separate water-only exposures. Individuals were collected at six different time points over a two-week period to detect and understand changes in metabolites over time. Metabolites were extracted from whole worms and polar metabolites were measured using GCMS. The major metabolite classes detected in worms after exposure to boscalid included amino acids and sugars. When exposed to boscalid the changes in metabolites became noticeable at 48-hours onward. Zinc and boscalid had different metabolomic trending variations that were observed after exposure. Further investigation of these results will be discussed regarding the usefulness of incorporating environmental metabolomics into biomonitoring programs, as changes in metabolites could be used as diagnostic indicators of contamination.

P-113	Metabolic effect of the OsCYP96B4 gene mutation in a novel semi-dwarf rice mutant investigated by NMR-based metabolomics and RNA-seq

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CO-AUTHORS: *Rengasamy Ramamoorthy, Shivshankar Umashankar, Srinivasan Ramachandran, Sanjay Swarup, Prakash P. Kumar*

Dwarfism and semi-dwarfism are among the most valuable agronomic traits in crop breeding, which were adopted by the "Green Revolution". Previously we reported a novel semi-dwarf rice mutant derived from the insertion of a single copy of Ds transposon into the gene OsCYP96B4. But the systems metabolic effect of the mutation is not well understood, which is important for understanding the gene function. Here, the differences in the metabolome and transcriptome between the semi-dwarf mutant, ectopic expression (ECE) and wild-type (WT) rice were investigated by NMR-based metabolomics and RNA-seq. Compared with WT, ECE of the OsCYP96B4 gene resulted in significant increase of ?-aminobutyrate, glutamine, and alanine, but significant decrease of glutamate, aromatic and branched chain amino acids, and some other amino acids. The ECE caused significant concentration increase of monosaccharides (glucose, fructose), but significant concentration decrease of disaccharide (sucrose); induced significant concentration changes of metabolites involved in nucleotide (adenosine, adenosine monophosphate, uridine) and choline metabolism (phosphocholine, ethanolamine). These metabolic profile alterations were accompanied with changes in the genes involved in GABA shunt, amino acid metabolism, shikimate-mediated secondary metabolism, glycolysis pathway, sucrose metabolism and nucleotide metabolism. Semi-dwarf mutant showed similar but less pronounced changes, especially in the transcriptome. The present study indicates that OsCYP96B4 gene mutation caused significant alteration in the GABA shunt, amino acid metabolism, shikimate-mediated secondary metabolism, glycolysis pathway, sucrose and nucleotide metabolism. It will provide essential information for the OsCYP96B4 gene function analysis and will serve as reference data for the development of new semi-dwarf mutants.

P-114	Metabolic reprogramming in PGPR-primed Sorghum bicolor in response to Colletotrichum sublineolum infection.

PRESENTING AUTHOR: *Ian Dubery, University of Johannesburg, South Africa*

CO-AUTHORS: *Fidele Tugizimana, Paul Steenkamp, Lizelle Piater, Nico Labuschagne*

Defence priming is a complex natural phenomenon that pre-conditions plants for enhanced defence against a wide range of pathogens. It thus represents a sustainable alternative / complementary strategy for protection against disease. However, a comprehensive functional and mechanistic understanding of various layers of priming events is still limited. Here, a non-targeted metabolomics approach was used to investigate metabolic changes in plant growth promoting rhizobacteria (PGPR)-primed Sorghum bicolor seedlings following Colletotrichum sublineolum fungal infection. At the 4-leaf growth stage, plants were treated with a PGPR strain, Paenibacillus alvei at 108 cfu/mL. Twenty four h following PGPR application, plants were inoculated with C. sublineolum (106 spores/mL). The infection was monitored over time: 1, 3, 5, 7 and 9 d.p.i.. Non-infected plants served as negative controls. Intracellular metabolites were extracted with 80% methanol-water and analysed by UHPLC-MS. The acquired data was analysed using different chemometric methods. These computed models revealed strong defence-related metabolic reprogramming observed in primed (compared to naïve) plants as early as 1-3 d.p.i.. Evaluation of orthogonal projection to latent structure-discriminant analysis (OPLS-DA) loading shared and unique structures (SUS)-plots uncovered that the differential stronger defence responses against the fungal infection observed in primed plants involved mostly enhanced levels of amino acids (phenylalanine, tyrosine, tryptophan), phytohormones (JA and SA conjugates, and zeatin), and defence-related components of the lipidome. Furthermore, other defence responses in both naïve and primed plants were characterised by a complex mobilisation of phenolic compounds and de novo biosynthesis of 3-deoxyanthocyanidin phytoalexins (apigeninidin, luteolinidin), apigenin, luteolin and related conjugates.

P-115	Metabolomics as a tool to study allelopathic interactions between Mediterranean plant species

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CO-AUTHORS: *Bernd Schneider*

Allelopathy plays a very important role in ecosystems. It is defined as any direct or indirect, harmful or beneficial effect of one plant on another through the production of chemicals released into the environment.1 The understanding of this phenomenon has been partially constrained, among other things, by the methods available to study the secondary metabolites involved. A new method based on metabolomics has been recently developed,2,3 and it is herewith applied to the study of allelochemicals from selected plant species of the Mediterranean region. Donor plant (Arbutus unedo, Myrtus communis, Medicago minima and Daphne gnidium) extracts were analysed by 1H and 2D NMR in order to define their chemical composition. They were tested for their phytotoxicity on a receiving plant species (Aegilops geniculata) on which morphological and metabolomics analyses were performed. Tests were carried out also with partially purified fractions and with the pure putative allelochemicals. The extracts of the four plant species showed a strong inhibitory activity on the receiving plant. NMR paired with multivariate data analysis of the receiving plant let to hypothesize the main metabolic pathways affected. Studies with the pure compounds confirmed in some cases the putative allelochemicals, while in other cases it was possible to determine the occurrence of synergistic effects. Some of the compounds were taken up and, in some cases, modified by the receiving plant. References 1. Rice E. L., Allelopathy, 1984 2. D'Abrosca B. et al., 2013. Phytochemistry 93, 27 3. Scognamiglio M. et al., 2014. Phytochemistry 106, 69

P-116	Defence-related metabolic reprogramming in Sorghum bicolor in response to Colletotrichum sublineolum infection

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Metabolic alterations of sorghum cultivars responding to Colletotrichum sublineolum infection, were investigated. Plants were inoculated with a fungal spore suspension (106 spores/mL) at the 4-leaves growth stage. The infection was monitored over time: 0, 3, 5, 7 and 9 d.p.i.. Non-infected plants were used as negative controls. Intracellular metabolites were extracted with 80% methanol-water. The extracts were analysed on an UHPLC system coupled to high-definition mass spectrometry and the acquired data were processed and analysed. Chemometric models indicated time- and cultivar-related metabolic changes that reflect defence responses to the infection. Metabolic pathway and correlation-based network analyses revealed a functional metabolic web, containing defence-related molecular cues to counterattack the pathogen's invasion. Components of this network are altered-metabolites from a range of interconnected metabolic pathways including phenylalanine metabolism, phenylpropanoid/flavonoid biosynthesis, riboflavin- and tryptophan metabolism; with the phenylpropanoid/flavonoid pathways being the central hub of the web. One of the key features of this altered metabolism was the accumulation of an array of phenolic compounds, particularly the 3-deoxyanthocynidin phytoalexins, apigeninidin and luteolinidin (and related conjugates), that exhibit fungitoxic properties to halt pathogen proliferation. The metabolic results were complemented by gene expression analyses that showed upregulation of defence-related genes, PR3, PR10, PAL, PPO and flavonoid 3'-hydroxylase (F3'H). Unravelling key characteristics of the biochemical mechanism underlying the sorghum–C. sublineolum interactions provided valuable insights with potential applications in crop protection. Furthermore, the study contributes to ongoing efforts towards a comprehensive understanding of the regulation of plant metabolism under biotic stress.

P-118	Callus tissues as model systems for studying host-pathogen interactions between New Zealand kauri and Phytophthora species.

PRESENTING AUTHOR: *Laura Raymond, Scion, New Zealand*

CO-AUTHORS: *Stefan Hill, Nari Williams*

The mighty kauri (Agathis australis), one of New Zealand's most iconic indigenous tree species is currently under serious threat from kauri dieback, a deadly disease caused by the pathogen Phytophthora agathadicida. Phytophthora, which translates from Greek as "plant destroyer" have been the cause of some of the most devastating widespread global epidemics, with perhaps the most notorious example P. infestans the cause of the 1840's Irish potato famine. To understand the mode of infection of Phytophthora, we have employed a systems biology approach covering a range of analyses from genes (transcriptomics) to pathology. As part of this study, metabolomics based on NMR and LC-MS has been implemented to understand chemical markers associated with the various infection stages. Complications can arise in analysis when dealing with real world trees that have not only Phytophthora, but a host of unknown other biotic and abiotic stressors impacting on the metabolome. To overcome this challenge and single out chemical markers for Phytophthora infection (either from the host or Phytophthora) plant callus tissue was cultured as model system for studying host-pathogen interactions. Kauri callus tissue was challenged with three species of Phytophthora known to infect kauri (P. agathidicida, P. cinnamomi, and P. multivora) and 1H NMR spectroscopy of aqueous extracted secondary metabolites was used to screen the validity of this approach to understand the infection process. Preliminary 1H NMR analysis indicate that the controls and Phytophthora infected callus tissues have statistically distinct chemical profiles and these differences correlate with the level of Phytophthora infection.

P-119	LIPIDOMICS APROACH TO IDENTIFY NOVEL SALINITY RESPONSE MECHANISUMS IN BARELY ROOTS

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CO-AUTHORS: *Dingyi Yu, Siria Natera, Ute Roessner*

Barley (Hordeum vulgare L.) is an essential food and brewing crop. As a glycophyte, it suffers substantial yield loss when grown under saline conditions. Relatively little is currently understood of salt stress perception and responses in plant roots, which involve complex changes at the physiological, metabolic, molecular, transcriptional, and genetic level. We aim to develop new tools to unravel how plants respond to the perception of salt stress. Evidence is accumulating that lipid signaling is an integral part of the complex regulatory networks in the responses of plants to salinity through modifications of membrane lipids, which occur through the activity of phospholipases, lipid kinases and phosphatases such as phospholipase D and diacylglycerol kinase that produce different classes of lipid and lipid-derived messengers. In addition, abiotic stress provokes enhanced production of reactive oxygen species, resulting in lipid modifications producing oxidized lipid species.. Initial analyses using lipidomics revealed that roots from tolerant and sensitive cultivars respond differently to salt stress. To investigate the modifications of lipids leading to root responses to salinity, we are using a combination of multiple approaches such as targeted and untargeted lipid analyses of barley roots. We also developed new lipidomics approaches using high-resolution mass spectrometry technologies to monitor the level of oxidized lipid species produced in barely roots due to salinity. Given the lack of fundamental knowledge of the lipids involved in signaling and metabolism under salinity stress, our results provide insight into novel mechanisms how barley roots respond to salt stress.

P-120	ENVIRONMENTAL METABOLOMICS PROVIDES INSIGHTS INTO PHYSIOLOGICAL RESPONSES OF SOUTHERN SAND FLATHEAD IN PORT PHILLIP BAY

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Port Phillip Bay is located on the central south coast of Victoria, Australia. It is a large urbanised marine embayment encompassing an area of roughly 1950 km2 with a coastline approximately 264 km; and hosts a population of over four million people on its catchment basin. The Bay receives waters from the Yarra River, discharges from sewage treatment plants, and petrochemical and agricultural inputs. It is home to a wide range of fish species, with seagrass beds used as nursery sites. The southern sand flathead (Platycephalus bassensis) are long-lived carnivorous ambush predators that have a sedentary, non-migratory lifestyle. They conceal themselves in fine sediments, are not strong swimmers and are believed to be representative of the area from where they are collected. Consequently they are considered a suitable bioindicator species for their local environment. The aim of this study is to use metabolomics to investigate responses of sand flathead to different environments in Port Phillip Bay. Two year old female fish were collected from five sites within the bay. Assessment of the fish for general condition showed differences between fish from urbanised/industrialised sites (Corio Bay, Hobsons Bay, Mordialloc) compared to fish from low population density areas (Sorrento and St Leonards). For the metabolomics analysis, livers were subjected to polar metabolite analysis using gas chromatography mass spectrometry. The PCA results showed that the samples from Sorrento separated from those of the other sites. Notably, fish from Sorrento and Mordialloc showed significant differences in amino acids, glycolytic and TCA intermediates.

P-121	Bisphenol S Exposure Modulate Macrophage Phenotype as Defined by Cytokines Profiling, Metabolomics and Lipidomics analysis

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CO-AUTHORS: *Zhi Tang, Jiacheng Yan, Zongwei Cai*

As an important structural analogue of bisphenol A (BPA), bisphenol S (BPS) has been used as alternative to BPA in industrialized production. However, the immunotoxicity of BPS remains poorly understood. As a critical model in inflammatory responses, macrophages are used to explore the immunotoxic potential and mechanisms of BPS at environmentally relevant concentrations in our study. Here, we are combining molecular toxicology and mass spectrometry (MS)-based global metabolomics and lipidomics study together to estimate the variation of cytokines profiling and metabolism characteristic following BPS exposure. Our results demonstrated that BPS exposure induced pro-inflammatory phenotype by activating the immuno-related cytokines which include TNF-?, IL-1? and IL-6, modulating metabolic pathways which include glycolytic, glutathione (GSH), sphingomyelin (SM)-ceramide (Cer), glycerophospholipids (GPs) and glycerolipids (GLs). These toxicological mechanisms are providing us with a deeper understanding of the critical role of metabolites and lipids reprogramming in immunotoxicity of BPS.

P-122	Identifying Early Urinary Metabolic Changes with Long-Term Environmental Exposure to Cadmium by Mass-Spectrometry-Based Metabolomics.

PRESENTING AUTHOR: *Gao Yanhong, Centers for Disease Control and Prevention of Guangdong Province, China, China*

CO-AUTHORS: *Lu Yonghai*

Cadmium (Cd) is a common environmental pollutant, and urinary Cd (UCd) is generally used as a marker of exposure; however, our understanding on the related urinary metabolic changes caused by Cd exposure is still not clear. In this study, we applied a mass-spectrometry-based metabolomic approach to assess the urinary metabolic changes in human with long-term environmental Cd exposure, aimed to identify early biomarkers to assess Cd nephrotoxicity. Urine samples from 94 female never smokers aged 44?70 with UCd in the range of 0.20?68.67 ?g/L were analyzed by liquid chromatography quadrupole time-of-flight mass spectrometry (LC-Q-ToF-MS) and gas chromatography?mass spectrometry (GC?MS). It was found that metabolites related to amino acid metabolism (L -glutamine, L -cystine, L -tyrosine, N-methyl- L -histidine, L -histidinol, taurine, phenylacetylglutamine, hippurate, and pyroglutamic acid), galactose metabolism (D -galactose and myo-inositol), purine metabolism (xanthine, urea, and deoxyadenosine monophosphate), creatine pathway (creatine and creatinine), and steroid hormone biosynthesis (17-?-hydroxyprogesterone, tetrahydrocortisone, estrone, and corticosterone) were significantly higher among those with a UCd level higher than 5 ?g/L. Moreover, we noticed that the level of N-methyl- L -histidine had already started to elevate among individuals with a UCd concentration of ?2 ?g/L. The overall findings illustrate that metabolomics offer a useful approach for revealing metabolic changes as a result of Cd exposure.

P-123	Metabolomics analysis of volatile organic compounds from a tomato core collection

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CO-AUTHORS: *Su-Yeon Kim, Jaeeun Song, Joon-Soo Sim, Bum-Soo Hahn*

The combination of volatile organic compound(VOC) plays an important role in the food and beverage industries. Based on the features of fruit weight, sweetness, peel color, and flesh color, we selected a tomato core collection constituted with 43 varieties. To investigate compositional differences that were involved in ripening of tomato, we analyzed VOCs by targeted-metabolomics. Among 43 tomato varieties, we identified 40 volatile metabolites from 27 groups of tomato fruits by comparing GC-TOF-MS profiles of each steam-distilled extracts. The VOCs observed in the majority of tomato varieties were trans-2-hexenal(42.4% of total VOCs) and hexanal(15.9%). Another abundant VOCs included ethylbenzene(6.8%), 6-methyl-5-hepten-2-one(3.6%), 1-hexanol(3.6%), 2-phenylethanol(3.5%), and 2-isobutylthiazole(3.3%). For the characterization of cultivar-specific VOCs, the metabolite profiles were subjected to principal component analysis. The component clusters revealed that the 2 Solanum lycopersicum varieties(WIR6473 and 19990913) showed high accumulation of terpenes, including farnesylacetone and trans-geranylacetone. The amounts of volatile alcohols, 1-hexanol and trans-2-hexen-1-ol, was highest in S. ceresiforme(IT297128). Interestingly, 4 wild-types demonstrated unique constituents of VOCs. S. galapagense(IT199485) contained trans-2-nonenal and heptanal, while S. pimpinellifolium(IT173722) maintained 1-octen-3-one, 1-heptanol, and linaool. Other wild-type varieties, S. peruvianum(IT173831) and S. lycopersicum(IT296530) showed high contents of 1-octen-3-ol, and nonanal, respectively. There was no clear relationship between VOCs and agricultural categories chosen for the construction of core collection. These results imply the possibility of VOC component indexing between diverse tomato core collections by GC-TOF-MS metabolomics.

P-124	Development of a GCMS-based Metabolomics Pipeline and its Application to an Insect Model

PRESENTING AUTHOR: *Joel Herring, Queensland University of Technology, Australia*

CO-AUTHORS: *Pawel Sadowski, Silvia Gemme, Anthony Clarke*

Tephritid fruit flies are the single most important horticultural pests of the Australian, Pacific, and Asian regions, being the major cause of 'grubs in fruit' and causing 100's of millions of dollars of crop damage annually (Fletcher 1987). Development of novel tools to understand the biology of these pests better is crucial to their eventual long-term and environmentally sustainable control (Clarke et al. 2011). Metabolomics is emerging as a convenient and relatively cheap way to validate the data found using techniques such as genomics and transcriptomics. This study aims to develop a GCMS-based (trimethylsilyl (TMS) derivatised) metabolite profiling pipeline using a low resolution instrument, Shimadzu GCMS TQ8040 and a high resolution accurate mass platform, the Thermo QExactive GC Orbitrap (QEGC) and apply it to the Queensland fruit fly (Bactrocera tryoni). The data corresponding to a different dietary regime was analysed using TraceFinder and Skyline software tools. The high resolution mass spectra obtained from the QEGC provided confident compound identification (via the Golm Database) when Tracefinder software was used for compound deconvolution while Skyline enabled compound quantitation using extracted ion chromatogram (XIC) data. This is the first time a metabolite profiling approach has been applied to this destructive pest.

P-125	Environmental Metabolic Footprinting (EMF) vs half-life: proposition of an integrative new proxy to evaluate pesticide environmental fate and impact

PRESENTING AUTHOR: *Cédric BERTRAND, CRIOBE - Université de Perpignan, France*

CO-AUTHORS: *Marie-Virginie Salvia, Amani Ben Jrad, Rahul Suryawanshi, Chandrashekhar Patil, Delphine Raviglione*

Pesticides are regularly used for diverse applications and are disseminated in the environment. These substances could be harmful and their presence must be monitored. To date, the half-life, t1/2, was often employed in order to study the environmental fate of pesticides. It can indicate whether or not the compound will persist in the environment. However, this value gives restricted information as it does not describe all the phenomena occurring such as the formation of by-products and the effect on biodiversity. A new approach, the "Environmental Metabolic Footprinting" (EMF)[1][2][3], was developed in the laboratory. It brings a new integrative proxy, the resilience time that reflects all the previously described phenomena. One other advantage appearing using this approach is that it permits studying the environmental impact of complex mixtures. No tools are available yet and they are required as biocontrol products are considered. This approach was tested within microcosms (soil, sediment) on botanicals and microbials (Bti ,insecticide). [1] C. Patil et al. (2016) EMF : a novel application to study the impact of a natural and synthetic ?-triketone herbicide in soil. Science of the Total Environment 566-567, pp. 552-558 [2] C. Patil et al. (2015) Metabolomics approach to evaluate fate and impact of natural herbicides in the environment. The 11th International Conference of the Metabolomics Society, San Francisco, (Poster). [3] C. Bertrand et al. (2016). Metabolomics approach to evaluate resilience of soil after treatment with natural herbicide. 13th International Conference on Protection and Restoration of the Environment, Mykonos , Greece (Oral).

P-126	Functional analysis of Buffalo grass (Stenotaphrum secundatum) expressing a novel gene conferring glufosinate-resistance

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CO-AUTHORS: *DANIEL DIAS, TREVOR STEVENSON*

Buffalo grass (Stenotaphrum secundatum) is a popular turfgrass species widely used in golf courses, athletic fields and home lawns. A serious problem affecting the quality of this turf is the infestation with annual weeds that requires extensive effort and investment to control. To simplify weed management, a transgenic herbicide-resistance (HR) turfgrass together with a broad spectrum herbicide affords cheap and efficient weed control. Recently, a novel gene sequence isolated from Alcaligenes faecalis was inserted into the Buffalo grass to provide resistance to the commercially available, broad spectrum herbicide, glufosinate. Molecular analyses of 4 transgenic events revealed a variation in gene copy number being corresponded with expression levels of this gene. In a glasshouse experiment, all of our HR grasses survived the glufosinate treatment at a commercial dose (0.5% v/v) over the wild types. Furthermore, the HR grasses with a greater insert gene copy numbers demonstrated lower glufosinate-induced visual injuries than the other HR grasses. To further assess the effects of the introduced gene on the molecular and physiological phenotypes, GC-MS based metabolite profiling was performed. Comprehensive analyses, both molecular and metabolomics confirmed the negligible effects of expression of the inserted gene upon plant metabolisms. Here, detailed analyses and supporting evidence will be presented.

P-127	Metabolomics reveals metabolic disturbance in adult mice after neonatal exposure to triphenyl phosphate and its metabolite

PRESENTING AUTHOR: *Dezhen Wang, China Agricultural University, China*

CO-AUTHORS: *Wentao Zhu, Yao Wang, Jin Yan*

The widespread application of organophosphates flame retardants (OPFRs) provides the opportunity for human exposure, which is raising great concerns. However, information on these chemicals' toxicological effects in rodents is limited. Here, we investigated the effect of triphenyl phosphate (TPP), one of the most widely used OPFRs, and its main metabolite diphenyl phosphate (DPP) on the endocrine systems and metabolic profiles after neonatal exposure from postnatal day 1 to 10 in ICR mice. Both TPP and DPP exposure advanced the opening of vagina, but did not induce significant uterotrophic response. In addition, low dose of TPP increase the body weight of male mice. Metabolic fingerprinting showed that low doses of TPP mainly disturbed lipid metabolism, while DPP and high doses of TPP impacted pyruvate metabolism and TCA cycles. Taken together, our observations show that TPP could affect endocrine systems and induced metabolic disturbances in adult mice.

P-128	Variation and Correlation Analysis of Carotenoids and Tocols in Pigmented Rice Cultivars

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CO-AUTHORS: *Soo-Yun Park, Sun-Hyung Lim, Sun-Hwa Ha, Jae Kwang Kim*

Correlations between the concentrations of various metabolites can be examined to gain information regarding metabolic associations. Gas-chromatography coupled with time-of-flight mass spectrometry (GC-TOFMS) and high-performance liquid chromatography (HPLC) were used to analyze the relationships between flavonoids, carotenoids , policosanols, sterols, and tocols in rice (Oryza sativa L.), including five black cultivars and two red cultivar. The metabolite profiles were subjected to data mining processes, including principal component analysis (PCA), Pearson's correlation analysis, hierarchical clustering analysis (HCA) and batch learning-self organizing map analysis (BL-SOM). We performed PCA to evaluate the differences among cultivars. PCA could fully distinguish between these cultivars. HCA of these metabolites resulted in clusters derived from common or closely related biochemical pathways. The distance among neurons within SOMs indicates the correlation between metabolites. Our results showed that carotenoids were correlated positively with tocotrienols. The metabolic profiling approach could be used as an alternative method to predict food quality and identify metabolic links in complex biological systems.

P-129	Quantification of de novo triacylglycerol synthesis through stable isotopic labelling during nitrogen deprivation in Chlorella sp

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CO-AUTHORS: *Damien Callahan, Stella Loke, Peter Beech*

Neutral lipids [e.g., triacylglycerol (TAG)] are high-energy storage molecules accumulated by some microalgae. The fatty acids (FAs) within TAGs can be converted to fatty acid methyl esters for biodiesel. It is well-documented that stress conditions can increase TAGs production in some microalgae. However, it was unknown how much of the newly made TAG was from newly fixed carbon or recycled carbon from within the cell. To determine the changes in carbon partitioning, the 13C stable isotope was added to the culture as bicarbonate, ensuring any newly fixed carbon would result in labelling of the FAs. To measure the FA labelling, a novel approach was applied using solid phase extraction to isolate the three main lipid classes followed by negative chemical ionisation gas chromatography mass spectrometry of pentafluorobenzl ester derivatives of the FAs. This technique allows intact FAs to be measured and hence the full isotopomer pattern can be determined. This novel approach enabled the amount of de novo FA production to be determined in the three key lipid classes. These data showed that, under nitrogen deprivation (ND), de novo FAs in TAGs were still being formed at three times the rate compared to nitrogen replete conditions. The de novo TAGs formed under ND were synthesised mostly from newly fixed carbon; however, there was some evidence that TAGs were also produced by recycling carbon from the pre-existing lipid pool. These data have great implications for optimising growth parameters towards maximising TAG synthesis while maintaining high rates of biomass production.

P-130	The impacts of Barley yellow dwarf virus titre on the wheat metabolome under elevated CO2

PRESENTING AUTHOR: *Simone Rochfort, Agriculture Research Victoria, Australia*

CO-AUTHORS: *Simone Vassiliadis, Kim Plummer, Kevin Powell*

Nuruk, a traditional Korean fermentation starter for brewing alcoholic beverages from starch, is a dough made from grains such as wheat, barley, or rice by various enzyme-releasing microorganisms. To investigate whether these microorganisms in Nuruk affect the quality of the alcoholic beverages produced, we conducted a foodomics study on three kinds of superior quality Korean traditional Nuruk, Omegigok, Baksuwhandongjugok, and Migok. These Nuruk were selected from 58 kinds of restored or commercial Nuruk through assessments of brewing properties and organoleptic characteristics. The foodomics study of each Nuruk as well as its alcoholic beverage products was performed through a combination of metagenomics and metabolomic approaches, involving various instruments such as GC-MS, CE-TOF-MS, and 1H NMR along with multivariate analyses. In addition, microbial communities were monitored during the fermentation process to identify correlative relationships between metabolites and microbes. Results revealed that the volatile metabolites of alcoholic beverages fermented using Nuruk are likely strongly affected by the microbial community in Nuruk, whereas the non-volatile metabolites are affected by Nuruk ingredients. In addition, even if Nuruk is contaminated by pathogenic microorganisms, these microbes are killed with the commencement of alcohol fermentation, and the microbial composition reverts to the normal, desirable Nuruk microbial composition. This metabolomic information and profile of each Korean traditional Nuruk will contribute to the understanding of the correlation between Nuruk and traditional alcoholic beverage quality as well as to the overall development of alcoholic beverages.

P-287	In-orchard metabolomics to understand plant responses to orchard management practises

PRESENTING AUTHOR: *Daryl Rowan, Plant & Food Research, New Zealand*

CO-AUTHORS: *Tony McGhie, Janine Cooney, Helen Boldingh, Duncan Hedderley, Annette Richardson*

Kiwifruit (Actinidia) are vigorous perennial vines requiring intensive management (canopy pruning, girdling and elicitor treatments) to maximise fruit yields and provide protection from the bacterial pathogen Pseudomonas syringae pv. Actinidiae. We ask; can we employ in-orchard metabolomics to understand and predict plant responses to these orchard management practises? We have adopted a combined LC-MS based metabolomics and targeted phytohormone analysis approach. Progress, and challenges, encountered in moving metabolomics into the orchard are discussed.

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O-93	MS-based Flavor Profiling of Okinawan Subtropical Plant Resources

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CO-AUTHORS: *Ning Wang, Miyako Kusano, Naoto Hirose, Makoto Takeuchi, Koji Wada*

Volatile flavors are complex mixture of aroma compounds in various concentration and intensity levels in organisms, particularly in plants. They make large effects on flavor perception and food taste, therefore to food quality. Comprehensive volatile flavor analysis by using gas chromatography-mass spectrometry (GC-MS) with selected ion monitoring (SIM) revealed discriminant chemical markers in volatile flavor component of Okinawan subtropical plant resources, including Hirami lemon and pineapple. The recorded total ion masses were hyphenated and used as dataset for discriminating genetic and seasonal diversities of these fruits. The unsupervised multivariate data construction via principal component analysis extracted specific qualifier ions. These are m/z 119, 134, and 135 corresponding to the mass spectrum of limonene and ?-terpinene and m/z 149 and 164 in that of methyl thymol as discriminant variables in the profiles of Hirami lemon, whilst m/z 57, 59, 74, 88, and 134 were found in the mass spectra of various esters in pineapple. The flavor profile constructions of these plant resources were further elucidated for their flavor distinctiveness using hierarchical clustering. The developed method was also applied to non-centrifugal brown sugars derived from sugarcane. The results revealed key discriminant ions for associated aroma compounds, including acetaldehyde (m/z 42 and 43), butanoic acid (m/z 55 and 73), acetic acid (m/z 60), and pyrazines (m/z 94, 108, and 122). The GC-MS/SIM-based volatile flavor profiling thus provide discriminative ion masses in aroma compounds of the Okinawan subtropical fruits as a potential "digital markers" that may impact the quality of plant resources and products.

O-108	Demonstration of the utility of biomarkers for dietary intake assessment; proline betaine as an example

PRESENTING AUTHOR: *Helena Gibbons, University College Dublin, Ireland*

CO-AUTHORS: *Charlotte Charlotte JR Michielsen Michielsen Michielsen, Milena Rundle, Gary Frost, Breige McNulty, Anne Nugent, Janette Walton, Albert Flynn, Michael Gibney, Lorraine Brennan*

There is growing interest in the development of metabolomics derived dietary biomarkers as objective measures of dietary intake. However, there is a dearth of studies demonstrating dose-response relationships and most importantly, use of metabolomics derived dietary biomarkers to estimate food intake is absent. The objective of this study was to demonstrate the use of dietary biomarkers in quantifying citrus intakes. NutriTech participants (n=50) consumed varying amounts of orange juice for three days over three consecutive weeks. Urine samples were collected and analysed on a 600-MHz Varian NMR spectrometer using the first increment of a NOESY pulse sequence. Calibration curves were developed to predict intakes and an independent cohort (n=565) validated the calibration curves. Proline betaine displayed a dose-response relationship to increasing orange juice in 24h and fasting samples (p<0.001). In a test set, orange juice intakes predicted using calibration curves displayed excellent agreement with true intake (bias: 43g: 24h, -18g: fasting samples). There were significant associations between predicted orange juice intake measured in 24h and fasting samples and true intake (r=0.710-0.919). Citrus intakes were predicted for the independent cohort with excellent levels of agreement with self-reported intake (bias 22g) and agreement improved following normalisation to osmolality (bias 4g). The development of calibration curves successfully enabled proline betaine to estimate citrus intakes in a large cross-sectional study. Furthermore, this was supported by demonstrating a dose-response relationship between proline betaine and orange juice intake. Expansion of this approach to other foods will be important for the development of objective intake measurements.

O-111	A muti-omics approach to understanding flavour-compound production in rice

PRESENTING AUTHOR: *Dara Daygon, The University of Queensland, Australia*

CO-AUTHORS: *Sangeeta Prakash, Ben Ovenden, Peter Snell, Mariafe Calingacion, Louise Forster, Mary Garson, Susan McCouch, Melissa Fitzgerald*

Screening for rice aroma quality has, in the past, focused solely on one fragrant compound, 2-acetyl-1-pyrroline (2AP), a strategy which is not sufficiently robust for large-scale analysis, and is not representative of the total aroma experience of rice consumers. We overcame these limitations by combining descriptive sensory evaluation with trained panellists, untargeted metabolite profiling of volatile compounds by two dimensional gas chromatography- time-of-flight mass spectrometry (GC ´ GC-TOF-MS), and a genome-wide association study (GWAS) using genotyping-by-sequencing. We determined the important metabolites that correlate with both pleasant and unpleasant aroma perception, and annotated these compounds through a combination of library matching and/or synthesis of authentic chemical standards. We further validated the impact of these metabolites on rice aroma in a set of rice varieties grown across three locations in Australia. Lastly, the genetic basis of the metabolites was determined through GWAS in a collection of 380 rice accessions. This approach lead to: (i) the identification of compounds that significantly impact rice aroma, and therefore, consumer preferences; (ii) the discovery of metabolites that provide evidences to unravel the biosynthesis 2AP; (iii) the identification of genetic regions and candidate genes associated with the production of several compounds; (iv) recognition of the effects of genotype-by-environment (GxE) interaction on the accumulation of indole, a repulsive-smelling compound whose production is found to be triggered by stress in some rice varieties. These results provide essential information in rice varietal development and in the optimisation of management practices in rice farming systems.

O-123	Detection of Novel QTLs for Metabolomic and Agronomic traits in Tea (Camellia sinensis)

PRESENTING AUTHOR: *Robert Koech, University of Pretoria, South Africa*

CO-AUTHORS: *Pelly Malebe, Christopher Nyarukowa, Richard Mose, Samson Kamunya, Zeno Apostolides*

Tea (Camellia sinensis) is popular beverage globally and has been demonstrated to have wide variety of pharmacological properties including; antioxidant, anti-inflammatory, anticancer, antidiabetic and neuroprotective effects. These health benefits associated with tea intake has led to high consumption of tea products. Tea quality is a complex and subjective trait and depends on its biochemical components. In this study, we have developed a genetic map using metabolomics couple with DArTseq platform on a segregating 251 F1 population derived from a reciprocal cross between two heterozygous tea cultivars (TRFK 303/577 and Gw Ejulu). The map consisted of 15 linkage groups that spanned 1162.80 cM with mean interval of 1.2 cM between markers. A total of 15 phenotypic traits were assessed in the two tea plant populations. Both interval and multiple QTL mapping revealed a total of 33 putative QTLs in the 15 LGs associated with tea quality at a significance genome-wide threshold of 5%. In total, six caffeine QTLs, 23 catechins QTLs, three theaflavins QTLs and one QTL for liquor brightness were detected. Of these 33, 13 major QTLs were identified for five traits in three main regions on LG01, LG03, LG11, LG12 and LG13. The population variability explained by each QTL was predominantly at moderate-to-high levels and ranged from 5.5% to 56.6%, with an average of 10.7%. The identification of QTLs that are linked to these markers at the seedling stage, and using Ultra-Performance Liquid Chromatography coupled with genetic markers would accelerate development of elite tea cultivars for marker-assisted selection.

O-128	A metabolomic approach to determine the influence of grape ripening, cultivar, vineyard site and vintage on wine composition

PRESENTING AUTHOR: *Leigh Schmidtke, Charles Sturt University, National Wine and Grape Industry Centre, Australia*

CO-AUTHORS: *Alain Deloire, Campbell Meeks, Guillaume Antalick, Katja Suklje, John Blackman*

Understanding the interactions of environmental and vineyard management influences on grape berry composition are the mainstay challenges for viticulturists and winemakers worldwide. Grape composition results from numerous interactions between cultivar growing conditions, water availability and the level of berry ripeness. A temporal and spatial investigation of berry, and subsequent wine composition and style was undertaken for two consecutive vintages in climatically diverse regions of Australia. Controlled triplicate fermentations of Shiraz and Cabernet Sauvignon grapes were undertaken. Harvest dates were determined at defined berry maturities using a sugar accumulation model to target three wine styles (Fresh, Intermediate and Mature). Sensory descriptive analysis of wines enabled different styles to be readily identified. Comprehensive profiling of grape composition, winemaking inputs, wine chemical and volatile composition, in addition to wine sensory scores generated nine individual data blocks for each wine. A metabolomic approach (ANOVA Multiblock Orthogonal PLS) was used to elucidate the impact of experimental factors (vineyard, region, vintage and grape harvest stage) for each cultivar. Loadings extracted from models with significant effects were subject to hierarchical cluster analysis (HCA) following rotation of model components to a consistent direction of effects levels in scores plots. The contribution of each data block to experimental factors, ANOVA designs and HCA assists in understanding the impact of grape berry ripening on Shiraz and Cabernet Sauvignon wine composition. Whilst vintage is a significant contributing factor, differences arising from grape harvest stage, region and vineyard management practices on grape berry and wine compositions could be determined.

O-220	Tomato juice consumption alters the human plasma metabolome

PRESENTING AUTHOR: *Jessica Cooperstone, The Ohio State University, United States*

CO-AUTHORS: *David Francis, Steven Schwartz, Janet Novotny, Earl Harrison*

Consuming a diet rich in tomatoes has been associated with a decreased risk for a number of different chronic diseases, including heart disease and cancers. Most attention has been paid to lycopene, the red pigment in tomatoes, as being responsible for these effects. However, studies where whole tomatoes are administered are often more efficacious than purified lycopene, suggesting other compounds from tomatoes may also play a role. The objective of this study is to understand how tomato supplementation affects the global metabolome of humans to better contextualize their health benefits. Tomatoes were grown specifically for this study and hot break processed to be shelf stable. Healthy non-smokers (n=35, BMIs from 18.5-30 kg/m2) consumed a low carotenoid diet for two weeks, and then all subjects consumed the same basal diet (designed for weight maintenance) for 4 weeks supplemented with 360 mL of one of three juices: high ?-carotene tomato juice (variety 97L97), high lycopene tomato juice (variety OH2461), or a calorie matched cucumber juice control. Methanolic extracts of plasma were analyzed comparing metabolite differences in individuals on these three diets. By employing UHPLC-QTOF-MS-based untargeted metabolomics, we were able to detect over 5,000 entities in plasma, and distinguish metabolite profiles based on diet using multivariate statistics and modeling techniques. An understanding of both exogenous and endogenous metabolomic changes in humans consuming tomatoes can generate testable hypotheses to understand ways in which this fruit may be exerting protective effects.

O-229	Inhibiting lipid oxidation using hydrophobic and hydrophilic natural antioxidants in an oil in water emulsion food system

PRESENTING AUTHOR: *Sara Ghorbani Gorji, University of Queensland, Australia*

CO-AUTHORS: *Mariafe Calingacion, Heather Smyth, Melissa Fitzgerald*

Oxidative instability of an oil in water emulsion (O/W) reduces the shelf life of the foods that contain these. Usually synthetic antioxidants are added to the O/W emulsion to prevent the development of rancidity. The objective of this work was to first elucidate the kinetic of oxidation in an O/W emulsion system stored for 92 days at 4 °C, 25 °C and 38 °C. Secondly, keeping in view the increasing demand for natural antioxidants, to investigate the antioxidant efficacy of hydrophobic and hydrophilic natural antioxidants in an O/W emulsion system stored at 38 °C for 60 days. The samples were analysed using both comprehensive two-dimensional gas chromatography (GC x GC) coupled with mass spectrometry (MS), and UPLC with MS and ion mobility. Descriptive sensory analysis of samples, combined with metabolomics provided new insights about the efficacy of both natural hydrophobic and hydrophilic antioxidants in an O/W emulsion system. The evolution of oxidation products was analyzed during storage, and very close relationships were found between the volatile compounds generated from fatty acids and the original oil composition in the fatty acids. This research is able to provide the food industry with natural antioxidants to use with validated capacity in the O/W emulsion.

O-272	Milk lipidomics: what we know and what we don't

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CO-AUTHORS: *Simone Rochfort, Ben Cocks*

Bovine milk contains 3-6% of fat, of which the dominant portion is in the form of triacylglycerols (TAG), while polar lipids including phospholipids and sphingolipids represent 0.5-1% of total fat. Several hundred of lipid species have been identified in bovine milk so far, making it the most complex material in nature in terms of lipid composition. Significant progress has been made in the past two decades in the identification of quantification of lipid species thanks to the advance in analytical tools especially high-resolution mass spectrometer. Currently milk lipid is characterised in two ways, i.e. global fatty acid profiling by GC and lipid species composition by LC-MS/MS; the former provides information on the abundance of each fatty acid, whereas the latter on the abundance and fatty acid composition of each lipid species. Using these analytical platforms, the influence of various genetic and environmental factors (such as cow breed, cow diet, stage of lactation and seasons) on lipid composition has been investigated. It is clear now that milk lipid composition displays remarkable cow-to-cow and seasonal variation, and is also prone to influence by animal diets. Milk lipidomic analysis still faces a number of challenges, such as determination of fatty acid position on glycerol backbone, chromatographic separation of TAG positional isomers and absolute quantification at the species level. As well as the peculiarity of milk lipid composition, the pros and cons of various LC separation techniques and MS methodologies for lipid structural elucidation and quantification will be illustrated in this presentation.

O-303	GC/MS-based metabolomics approach for the discrimination of Indonesian specialty coffee

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CO-AUTHORS: *Tomoya Irifune, Yusianto Yusianto, Eiichiro Fukusaki*

Coffee is one of the most consumed beverages in the world and there is an increasing global trend for consumption of high quality coffee, such as specialty Arabica and fine Robusta. Development of single origin coffee is one of the most important strategies to maintain coffee quality, grade and high cupping score. Indonesia is the second largest specialty Arabica exporter in the world with 150,000 tons export per year. In addition, Indonesia has over a dozen variety of specialty coffees from different geographical origins such as Java, Sumatera, Bali and Toraja coffee that are renowned in the global specialty coffee market. Despite its profitable prospect and high demand in the market, there is no reliable and standardized method for discrimination of Indonesian specialty coffee. In this study, non targeted GC/MS-based metabolomics approach was used to classify Indonesian high quality coffee based on geographical origin. Extracts of 16 green and roasted coffee beans (Coffea arabica and Coffea canephora) from different cultivation areas in Indonesia were analyzed and subjected to principal component analysis. Ten Specialty Arabica coffee from 7 cultivation areas and five Fine Robusta from 3 cultivation areas were used to further classify Indonesian coffee according to origin of cultivation. The result from this study demonstrate that the species and origins can be rapidly discriminated by evaluating the major metabolites of green coffee beans using GC/MS-based metabolite profiling. This is the first report to address the classification of Indonesian specialty coffee using metabolomics approach.

O-377	Estimation of dietary intake; comparison of a dietary biomarker and a 4-day food diary

PRESENTING AUTHOR: *Lorraine Brennan, UCD Institute of Food and Health, Ireland*

CO-AUTHORS: *Xiaofei Yin, Helena Gibbons*

To date, there is a dearth of data comparing the performance of dietary biomarkers to self-reported estimates of intake. The objective of this study was to compare the estimation of chicken intakes using the chicken biomarker guanidoacetate and chicken intakes reported in a 4-day food diary. Previously we identified guanidoacetate as a chicken biomarker using NMR based metabolomics. From the urinary guanidoacetate concentrations a calibration curve was built. Urine samples collected in the National Adult Nutrition Survey (NANS) (n=565) were analysed by a 600-MHz Varian NMR spectrometer and guanidoacetate was quantified. Chicken intake for NANS participants was determined using the biomarker data. Bland and Altman analysis evaluated the agreement between reported intake from NANS and intake from the biomarker data. A calibration curve was developed using urinary guanidoacetate concentrations ranging from 1.47 to 3.66 mM. Using this calibration curve (Y=1.08e-02X+4.96e-01, Y is urinary guanidoacetate (mM) and X is chicken intake (g)) and NANS participants' urinary guanidoacetate levels (concentration ranges of 0.07 to 3.13 mM) chicken intake was calculated for the NANS participants. Bland and Altman analysis revealed that the 95% limits of agreement of measurement differences ranged from -125 to 64.37 g/day, and the mean bias was -30.31 g/day demonstrating excellent agreement between reported and calculated chicken intake. This study demonstrates that the chicken biomarker performed as well as the 4-day food record in determining intake. This opens the possibility for the biomarkers to aid dietary assessment in future studies and marks a significant development for metabolomics derived dietary biomarkers.

O-442	A metabolomics approach to investigating red wine mouth-feel

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Wine mouth-feel is defined as tactile sensations felt inside the mouth when tasting wine or after expectoration. One important mouth-feel sensation often used when describing wines is "body". The term body is often used interchangeably with viscosity which is logical given the numerous studies that have found significant correlations between perceived and physical viscosity measurements. Previous studies have found a number of small molecules present at relatively low concentrations in wine also correlate with perceived viscosity, indicating there may be more to wine body than rheology. In this study 48 Pinot noir wines from two vintages and several geographic regions were subjected to sensory and chemical analysis. An expert panel of wine professionals assessed the body of the wines as well as a number of other sensory attributes. The non-volatile profile of the wines was analysed by reverse phase UPLC coupled with a Thermo Scientific Q-Exactive Orbitrap mass spectrometry system utilising polarity switching. Accurate mass data were acquired in centroid mode and processed using MZmine. Multivariate statistical analyses of the non-volatile profiles in combination with perceived body ratings, as scored by the expert panel, showed that a number of different features correlated with wine body and were driving the models. MSn experiments identified several candidate biomarkers in the wines that may contribute to perceived body. This study provides a platform to advance knowledge about how small molecules may influence wine mouth-feel.

O-461	Dietary whole grain modifies microbiota and enhances production of novel compounds associated with energy metabolism

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Epidemiological evidence suggests that diet rich in whole grains reduces the risk of lifestyle-related chronic diseases including cardiovascular diseases and type 2 diabetes. Molecular mechanisms responsible for the beneficial health effects are not well known, however, key contributor is the phytochemical and fiber dense bran that is maintained in whole grain based dietary products. The bran-derived components may have biological role themselves, and also via the colonic microbiota, since the role of diet in shaping the composition of microbiota has become evident, which in turn is an essential contributor to our health. Here we present a study with multiple metabolite profiling assays on a range of experiments involving in vitro, animal, and human based studies, and describe a novel group of compounds that are produced endogenously and by the colonic microbiota. We show that following the bran-induced shift in the composition of the microbiota these compounds are elevated in metabolically active organs in mice. Likewise, several of these compounds were increased also in humans after whole grain rich dietary interventions. Furthermore, we suggest their potential bioactive role, via demonstrating their implications in energy metabolism in cultured neonatal mice cardiomyocytes. Taken together, these compounds are one of the possible mediators for the beneficial health effects of whole grain rich diets.

O-464	Targeted metabolomics to provide insights into the role of trace sulfur compounds in wine aroma and quality

PRESENTING AUTHOR: *Vilma Hysenaj, Metabolomics Australia, The Australian Wine Research Institute, PO BOX 147, Glen Osmond, 5064, Australia*

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Volatile sulfur containing compounds (VSCs) play a significant role in the determination of the characteristic flavours and off-flavours of many foods and beverages. VSCs have been largely studied in wine because of their detrimental impact on wine aroma. In wine VSCs such as hydrogen sulfide (H2S), methanethiol (MeSH) and ethanethiol (EtSH) contribute to unwanted so called 'reductive aromas'. These compounds are responsible for imparting wine aromas such as egg, vegetable, burnt, rotten and struck flint. Understanding the formation and the fate of these compounds during fermentation and storage of wine is crucial as they negatively contribute to wine aroma and may also negatively impact wine quality. Measurement of these VSC by established GCMS methods can be tedious, time-consuming and often not reproducible due to the highly reactive nature of these compounds. To address these shortcomings we developed a fast and accurate UHPLC-MS/MS targeted derivatisation method that accurately quantifies thiols and disulfides in wine. This method was established to quantify the three main thiol compounds associated with 'reductive aromas' namely H2S, MeSH and EtSH, as well as thiols such as benzyl mercaptan which imparts aromas of struck flint that is an important component of minerality and other wine style-related characters. An additional five VSC's associated with 'reductive aromas' are also included in this method. Here we present an accurate and robust targeted metabolomics method able to monitor a wide range of thiol and disulfide compounds in wine. The method development, validation and its application for wine will be discussed.

O-497	Edibilomics

PRESENTING AUTHOR: *Hanne Christine Bertram, Aarhus university, Denmark*

CO-AUTHORS:

Diet contributes to shape our health. This fact receives increasing attention, and there is consequently a vast interest in developing efficient strategies to elucidate how the intake specific dietary components influences human health, and to unravel the underlying mechanisms by which they exert these effects. Metabolomics provides a useful tool for examining the endogenous response to intake of specific dietary components. By metabolomics analyses of a variety of samples such as blood, urine, feces and tissues, it may be possible to disentangle the way that a specific dietary component exerts effects on endogenous metabolism, on the activity of the gut microbiome and on host-gut microbiome interactions. In addition, metabolomics is also an efficient tool for examining how intake of specific dietary components is reflected the presence of exposure markers and thereby represents a pillar in biomarker discovery and biomarker establishment in nutrition research. Here examples of edibilomics: the application of metabolomics in nutrition research, will be presented.

O-500	Metabolomics tools and approaches for characterising flavours, phenolics and quality markers in grapes and wine

PRESENTING AUTHOR: *Markus Herderich, The Australian Wine Research Institute, Australia*

CO-AUTHORS: *Vilma Hysenaj, Esther Kristianto, Luca Nicolotti, Natoiya Lloyd*

The diverse chemical composition, unique sensory attributes and perceived quality of a wine result from combining 100s of metabolites of grapes, yeast and bacteria, and oak wood. So far only a relatively small proportion of secondary metabolites present in vines and grapes has been extensively studied; yet hundreds of molecular features can be detected in a comprehensive characterisation of a grapevine's or wine's metabolome. It remains to be established which of the many "known unknown" compounds may impart sensory effects in wine, are potentially suitable as quality markers, or could play a role in human nutrition as bioactive agents. Non-targeted metabolomics approaches therefore can provide a much greater understanding of how vines, yeast and bacteria, together with grape growing and winemaking practices, shape the composition and sensory properties of a wine. Notably, metabolomics approaches can also be applied to characterise a wine's metabolome and the complex chemical reactions in wine that are influenced by changes to grape processing and winemaking, or result from oxidation of wine compounds, acid hydrolysis and other chemical reactions during storage, transport and wine ageing. In this presentation key aspects of experimental design, method development and validation, and data analysis for successful metabolomics applications in grape and wine research will be discussed, with focus on the analysis of volatile trace metabolites and aroma compounds, non-targeted profiling of non-volatile polyphenols, and analysis of resveratrol metabolites in human plasma.

P-131	Rapid Evaporative Ionization Mass Spectrometry For High-Throughput Screening In Food Analysis: The Case of Boar Taint

PRESENTING AUTHOR: *Sara Stead, Waters Corp, United Kingdom*

CO-AUTHORS: *Renata Jandova, Kaat Verplanken, Christof Van Poucke, Lynne Vanhaecke*

Increasing awareness on animal welfare has led to a European Treaty announcing a voluntary ban on the surgical castration of piglets by 2018. An alternative to surgical castration is rearing of entire males, however, the main setback is the possible occurrence of boar taint, an off-odour caused by the release of androstenone (AEON), skatole (SK) and indole (IND) when the meat is heated. To prevent adverse consumer reactions, there is an urgent need for rapid methods allowing detection of boar taint at the slaughter line. Rapid evaporative ionization mass spectrometry (REIMS) has been used as an emerging technique to develop a predictive model for accurate high-throughput identification of boar taint in pig adipose tissue. A first of its kind step towards achieving at-line classification of boar carcasses. Pig adipose tissue was sampled using the iKnife handheld sampling device, which was connected directly to a Q-TOF mass spectrometer equipped with REIMS source. Untargeted mass spectrometric profiling in both negative and positive ionisation mode of pig neck fat samples enabled the construction of a predictive model for the classification of carcasses in boar taint positive or negative groups. The predictive model showed high accuracy (>93%) and a very low false positive and false negative rate (alpha and beta-error ? 5%), demonstrating the potential and applicability of this model on pig carcasses. The REIMS technique delivers a result within 10 seconds from sampling to result, this technique guarantees point-of-control analysis, which represents a major step forward in high-throughput screening of aberrant pig carcasses.

P-132	Effects of feeding frequency on the polar metabolite profile of human milk

PRESENTING AUTHOR: *Karl Fraser, AgResearch, New Zealand*

CO-AUTHORS: *Hanna Lagstrom, Johanna Makela, Shikha Pundir, David Cameron-Smith, Nicole Roy*

Human milk is the primary source of nutrition for infants and is recognised as the gold standard for feeding new-born full-term healthy infants. It delivers the nutrition necessary to support the growth and development of term infants during the first six months of life, and thus is rich in normal nutrients such as carbohydrates, fats, proteins, minerals and vitamins. It also contains an array of biologically-active constituents, including antimicrobial substances, growth factors, cytokines, immunoglobulins and specific immune cells. The analysis of human milk composition is complicated by many factors, including the changing nutritional composition of the milk: within a single feed; during the day; in response to maternal diet; and over the course of the lactation period. Thus the frequency of daily lactations will likely affect the metabolic composition of the milk. We present here untargeted metabolomics profiling of polar metabolites in human milk using hydrophilic interaction liquid chromatography MS (HILIC-MS) performed on a cohort of almost 800 women participating in the STEPS (Steps to healthy development) study in South-West Finland. Interrogation of the resulting HILIC-MS data matrix using feeding frequency classifications of either partial (i.e. infant supplemented with formula feeding) or full-time feeding by orthogonal partial least squared discriminant analysis revealed considerable differences in metabolite concentrations. For example, the tetrasaccharide stachyose and carnitine levels were both significantly (FDR<0.05) elevated in the partial feeding group. This demonstrates the value of metabolomics profiling to evaluate compositional differences within a highly complex sample matrix such as human milk.

P-133	Simpler variable selection for lipidomics using multilevel statistical tools

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CO-AUTHORS: *Yann Guitton, Sébastien Déjean, François Bartolo, Estelle Martineau, Bruno Le Bizec, Patrick Giraudeau, Gaud Dervilly-Pinel*

Metabolomic -and by extent lipidomic- studies generate large amounts of data, which require tailored statistical methods. Multivariate statistical analyses, such as Principal Component Analysis (PCA) for data exploration, or Partial Least Square Discriminant Analysis (PLS-DA), for supervised analysis are therefore commonly used [1]. However, when the experiment design includes repeated measurements (for instance several measurements acquired on the same individual at different time points), too few available exploratory solutions enable dealing with such characteristic, which generates biological noise and hinders further interpretation. The so-called multilevel approaches, as included in the mixOmics R package, make an efficient use of these repeated measurements and after subsequent multivariate analysis, better reveal the differences between sample groups and relevant variables [2-3]. We will demonstrate that a two-factor multilevel approach, followed by PLS-DA, can greatly simplify the interpretation of the results from the statistical treatment of lipidomic data acquired with multiple analytical methods. We will present a simple and efficient solution for relevant variable selection. The strategy has been efficiently applied in a public health related project to highlight biomarkers of chemical exposure in food producing animals on two data sets respectively acquired by Mass Spectrometry and multidimensional Nuclear Magnetic Resonance fingerprinting. A distinct discrimination between the sample groups was obtained, allowing a simple selection of the relevant variables, thus highlighting potential biomarkers.

P-134	Impact of carbon source and sulphur-amino acids on microbial-mediated volatile organic compounds released profile: Case study of a psychotropic food spoilage bacterium in a model system

PRESENTING AUTHOR: *Mohsen Zareian, University of Otago, New Zealand*

CO-AUTHORS: *Patrick Silcock, Phil Bremer*

Proton transfer reaction mass spectrometry complemented with GC–MS was used to investigate temporal changes in volatile organic compounds (VOCs) released profile of the psychotropic food spoilage bacterium, Pseudomonas fluorescence. Due to the highly heterogeneity and complexity of food matrices and the challenging task to identify characteristic volatile markers as spoilage indicators, a model system was designed to investigate a typical food spoilage scenario that frequently occurs in refrigerated foods. Basal medium (Vogel's) was supplemented with glucose (0 or 0.5 % w/v) and/or sulphur-amino acids i.e. cysteine (Cys, 0 or 1 mM) and/or methionine (Met, 0 or 1 mM). Met and Cys influenced the VOCs fingerprints produced particularly in terms of S- derivatives. Principal component analysis showed that the separation of compounds was based upon a combination of Met and time. Met derived the most variation of the compounds and the loading plot indicated that m/z 35 (H2S), m/z 95 (dimethyl disulfide), m/z 97 (dimethyl furans), m/z 63 (dimethyl sulfide) and m/z 49 (methanethiol) derived the most significant variation. In contrast, the fingerprint of the VOCs evolved from glucose and Cys revealed a different distribution pattern. Supplementation of Met to medium, containing glucose, derived further variation in the volatiles indicating that an additional cluster of compounds exist. Multivariate cluster analysis identified a distinct group of S-derivatives (m/z 63, m/z 95 and m/z 97) being clustered with the highest similarity percentage. Detailed information on VOCs release profile, especially of S-derivatives, can potentially be used as food spoilage indicators.

P-135	Estimating population food consumption and dietary status using wastewater based epidemiology

PRESENTING AUTHOR: *Phil Choi, University of Queensland - Queensland Alliance for Environmental Health Science, Australia*

CO-AUTHORS: *Jake O'Brien, Ben Tscharke, Sharon Grant, Rachel Mackie, Kevin Thomas, Jochen Mueller*

Estimating population dietary status using wastewater based epidemiology Wastewater is a unique repository of chemicals excreted by humans. Therefore, wastewater analysis can provide information regarding the chemicals consumed by humans in a given wastewater catchment. This approach, termed wastewater based epidemiology, has been used to estimate the consumption of drugs in a multitude of cities throughout the world. Here, we present our previous findings which used wastewater based epidmiology to show differences in alcohol consumption among different cities around the world, and during different days of the week. We also present food metabolites, food constituents and other nutrients which are currently under assessment to be used as biomarkers to estimate dietary status through wastewater analysis. Challenges involved in developing accurate models to estimate food consumption and nutrient intake from wastewater analysis are also discussed. Once developed, wastewater based epidemiology could serve as a novel method to objectively assess spatial and temporal dynamics in food consumption and dietary status at a population level.

P-136	Determining the geographical origin of beef using UPLC-Orbitrap-MS/MS based lipidomics

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CO-AUTHORS: *Ka-Yi MAN, Chi-On CHAN, Ka-Hing WONG, Kevin Wing-Hin KWOK*

Traceability of food origin is one of the most important public health issues for governmental authorities and food industry, especially for net food importing countries and cities. For consumers, the interest in origin of beef has increased in recent years. However, nutrition related tracing method of beef is absence. This study aims at using lipidomics approach to examine the geographical origin of beef by Ultra-Performance Liquid Chromatography coupled with electrospray ionization-Orbitrap-Mass Spectrometry (UPLC-ESI-Orbitrap-MS). Beef samples of total 39, including 8 samples with organic label from three different geographical origins (Australia, United States and Japan), were collected from the local market. In the partial least squares-discriminant analysis (PLS-DA) of the (–) UPLC-ESI-MS data, a clear distinction between the cluster of three different geographical origins was observed in the score plot. This indicated that the geographical origin is the dominant factor to affect the metabolite profiles of beef, specifically the phosphoethanolamine (PE) and phosphatidylserine (PS) group. In addition, the cluster of organic beef was well separated from conventional beef in PLS-DA of both (+) and (–) UPLC-ESI-MS data. This indicated that the metabolite profile of organic beef was different from the other groups. Multivariate statistics revealed that the level of diglyceride (DG) and PE group in organic beef was significantly higher than conventional beef, and the level of triglyceride (TG) is opposite.

P-137	Influence of yeast and lactic acid bacterium on the constituent profile of soy sauce during fermentation

PRESENTING AUTHOR: *Risa Harada, Department of Biotechnology, Graduate School of Engineering, Osaka University, Japan*

CO-AUTHORS: *Masanobu Yuzuki, Kotaro Ito, Kazuki Shiga, Takeshi Bamba, Eiichiro Fukusaki*

Soy sauce is a one of the most popular Japanese traditional seasonings. During the soy sauce production, firstly koji mold is inoculated into steamed soybean and roasted wheat (soy sauce koji making). Then koji is mixed in salt solution, and this mixture (called "moromi") is fermented by lactic acid bacterium (LAB) and yeast. Soy sauce contains various constituents, such as amino acids, saccharides, organic acids and volatiles that are metabolized by these microbes. Although constituents relate to soy sauce quality, the investigation of the constituent profile during fermentation is difficult due to the complexity of the constituent changes. Therefore, we applied metabolomics to soy sauce fermentation to understand the influences of each microbe on the constituent profile. We investigated changes in low molecular weight hydrophilic and volatile compounds using gas chromatography/mass spectrometry (GC/MS)-based non-targeted metabolic profiling. In this study, we analyzed four types of soy sauces considering the influence of these microbes (LAB+Yeast, LAB only, Yeast only, none). Through the statistical analysis of the constituent profile, we found constituents such as saccharides and amino acids which characteristically change in each fermentation phase. Additionally, our results suggested a novel finding that LAB affected the production of several constituents such as cyclotene, furfural, furfuryl alcohol and methional. This is the first study on the investigation of constituent changes based on the influence of microbes during soy sauce fermentation using metabolomics analysis. This aspect would be useful in the future to improve soy sauce quality by elucidating relationship between constituents and microbes.

P-138	Dissecting the Health Benefits of Red Wine Extract of Onion by Mass Spectrometry-Based Metabolomics Approach

PRESENTING AUTHOR: *Pui-Kin So, The Hong Kong Polytechnic University, Hong Kong*

CO-AUTHORS:

Onion is widely used in functional food formulations because of its multiple beneficial health effects, including antioxidant, anti-allergic, anti-obesity, anti-diabetic, anti-microbial effects, etc. On the other hand, red wine is also widely recognized to confer a wide range of health benefits. e.g., reducing risk factors of cardiovascular disease, anti-cancer, anti-inflammation, etc. Red wine extract of onion (RO) has been unofficially used to promote health and is emerged as a potential functional food formulation. RO was recently found to exert better cardioprotective effects than red wine because of its higher effectiveness in suppression of cholesterol level. So far, there was no study investigating the relationship between the chemical composition and health benefits of RO. In this study, we apply LC-MS to dissect the chemical-function relationship of RO by examining the chemical composition difference between RO, red wine (RW), onion extract (O) and mixture of RW and O (RWO). Our results showed that RO contained significantly higher level of antioxidant chemicals, e.g., quercetin and isorhamnetin derivatives and S-methyl cysteines, and other bioactive compounds, e.g. S-lactoylglutathione involving in detoxification, than RW, O, and RWO. For example, RO was found to contain up to 30 folds more quercetin derivatives than O and RWO, revealing that red wine could assist the extraction of bioactive components from onions during preparation of RO. These data provide understanding on the nutritional and functional value of RO on chemical composition basis and insight into further optimization of the functional formulation.

P-139	PERENNIAL RYEGRASS TOXICOSIS: EFFECTS OF THE INDOLE DITERPENOID MYCOTOXINS ON MOVEMENT AND METABOLISM IN A MOUSE MODEL

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The mycotoxin lolitrem B is found in endophyte-infected perennial ryegrass (Lolium perenne) and is present in around 90% of ryegrass pastures in Australia and New Zealand. Ingestion causes a neurological syndrome in grazing livestock called Perennial Rye Grass Toxicosis (PRGT) (Cunningham, 1959; Combs et al., 2014) where clinical signs include hyperexcitability, muscle tremors, ataxia ("staggers") and, in severe cases, clonic seizures and death (Combs et al., 2014). It remains unclear whether these neurological signs result only from the blockade of large conductance potassium channels (BK channels) by indole diterpenoid toxins, primarily lolitrem B (Munday-Finch SC., 1997; Imlach, 2011). Furthermore, the toxicity of biosynthetic pathway intermediates of lolitrem B is also largely undefined. To address these questions, lolitrem B and several intermediates were purified from infected perennial ryegrass. Using a time-series analysis, the effects on movement in dosed mice were quantified for lolitrem B and related compounds. Animals exposed to lolitrem B showed a significant increase in tremor and decreased ability to maintain stability during accelerated rotarod testing, while the other metabolites showed varied effects. Metabolic profiling of the mice tissues and serum were undertaken for animals exposed to lolitrem B at key time points. This study suggests the possible mechanism and quantity of residual lolitrem B in tissue samples. Characterization of clinical signs and metabolic changes associated with exposure to the family of indole diterpenoid toxins present in perennial ryegrass will provide useful information for generation of animal safe grass varieties for the dairy and meat industries.

P-140	M?nuka honey markers: linking nectar metabolites to honey authentication

PRESENTING AUTHOR: *Nigel Joyce, Plant & Food Research, New Zealand*

CO-AUTHORS: *John van Klink, Bruce Smallfield*

Poster TITLE: M?nuka honey markers: linking nectar metabolites to honey authentication John van Klink, Bruce Smallfield and Nigel Joyce. In New Zealand, m?nuka flowering supports a thriving and valuable honey industry, but is threatened by increasing supplies of fraudulent products. Our studies on field-sourced pure m?nuka honey confirmed several compounds that may be useful as biomarkers of genuine m?nuka honey including: o-methoxyacetophenone, the major headspace volatile by GC-MS, and phenyllactic acid, leptosperin and lepteridine by LC-MS. We have also been studying m?nuka nectar production in order to understand its regulation. Phytochemical analysis of nectar sampled from varying flower development stages was profiled in relation to phenotypic characteristics using a metabolomics approach. The expression of >400 compounds over six flower development stages was reviewed. The concentration of dihydroxyacetone, the precursor to the main m?nuka honey bioactive (methylglyoxal), followed the same pattern as total sugars. The key phytochemical markers leptosperin and lepteridine also followed this pattern through flower development, suggesting that their biosynthesis was intimately linked to nectar production. These data provide a baseline for studying m?nuka honey marker compounds and plant biosynthesis.

P-141	Unravelling the hidden potential of fruit/vegetable by-products

PRESENTING AUTHOR: *Ninna Granucci, University of Auckland, New Zealand*

CO-AUTHORS: *Philip Harris, Silas Villas-Boas*

New social and environmental concerns are challenging the current agri-food systems towards more sustainable practices from production to distribution. Therefore, the focus of this study was to build a more comprehensive picture of fruit and vegetable by-product composition to determine their potential for bioconversion and/or bio-refinery processes. The gross and fine chemical composition of three main fruit and vegetable by-products produced by New Zealand's juice and beverage industries (apple, orange and carrot pomaces) were assessed using a wide range of mass spectrometry based techniques, including GC-MS, LC-MS and ICP-MS. We were able to analyse over 300 different chemical components present in these substrates, including sugars, amino and organic acids, fatty acids, pesticide residues, minerals, protein amino-acid composition, in addition to basic nutritional profile (e.g. total protein, fat, soluble sugars, dietary fibres, phenolics, etc.). Overall, all by-product showed high levels of dietary fibres formed mainly by beta-1,4-glucans, and soluble sugars (fructose and glucose). However, most substrates presented low protein and amino acid content. Interestingly, around 20% of the total fat content in all substrates were formed by polyunsaturated fatty acids, including omega-6 and omega-9 fatty acids. Potassium, calcium, and phosphorus were the most abundant minerals, with carrot pomace showing the highest concentrations (15, 5, and 1.5 mg/g respectively). Carrot pomace also showed the highest sodium levels among all substrate tested (above 3mg/g). Therefore, despite considerable difference in composition between different by-products, our study clearly demonstrates that these usually wasted food grade substrates have great potential for bioconversion into high value-added products.

P-142	Foodomics reveals the roles of Korean traditional Nuruk in brewing technology

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Nuruk, a traditional Korean fermentation starter for brewing alcoholic beverages from starch, is a dough made from grains such as wheat, barley, or rice by various enzyme-releasing microorganisms. To investigate whether these microorganisms in Nuruk affect the quality of the alcoholic beverages produced, we conducted a foodomics study on three kinds of superior quality Korean traditional Nuruk, Omegigok, Baksuwhandongjugok, and Migok. These Nuruk were selected from 58 kinds of restored or commercial Nuruk through assessments of brewing properties and organoleptic characteristics. The foodomics study of each Nuruk as well as its alcoholic beverage products was performed through a combination of metagenomics and metabolomic approaches, involving various instruments such as GC-MS, CE-TOF-MS, and 1H NMR along with multivariate analyses. In addition, microbial communities were monitored during the fermentation process to identify correlative relationships between metabolites and microbes. Results revealed that the volatile metabolites of alcoholic beverages fermented using Nuruk are likely strongly affected by the microbial community in Nuruk, whereas the non-volatile metabolites are affected by Nuruk ingredients. In addition, even if Nuruk is contaminated by pathogenic microorganisms, these microbes are killed with the commencement of alcohol fermentation, and the microbial composition reverts to the normal, desirable Nuruk microbial composition. This metabolomic information and profile of each Korean traditional Nuruk will contribute to the understanding of the correlation between Nuruk and traditional alcoholic beverage quality as well as to the overall development of alcoholic beverages.

P-143	Global Untargeted Metabolomics Approach to Reveal Metabolic Shifts during Postharvest Cold Storage of 'Kinnow' Mandarin

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Fruit metabolism continues at an inhibited rate during postharvest cold storage, leading to significant changes in fruit quality attributes generally measured as quantification of a few metabolites having nutritional and flavour importance. Mass-spectrometry based global untargeted metabolomics is an approach to gain comprehensive coverage and insight into the fate of thousands of metabolites in a biological system. To study the metabolic shifts during cold storage of 'Kinnow' mandarin, commercially mature fruit were stored at low temperature (5°C) for 8 weeks and sampling was conducted at weekly intervals. Methanolic (80%) extracts of juice were injected into an UHPLC-QTOF mass spectrometer operated in an independent data acquisition mode enabling generation of TOF-MS and MS/MS data. Mass spectral output analysis involved peak alignment, normalization against an internal standard, and then unsupervised and supervised multivariate analyses using Analyst™, PeakView™ and MarkerView™ software. About 8000 features for each data set were detected in the mass range of 100-1000 m/z. Following data pre-processing, multivariate statistical analyses reflected significant metabolic shifts during cold storage for 8 weeks. Clustering of fruits into three major groups was possible: early-(0-2 weeks), mid- (3-5 weeks) and late- (6-8 weeks) stages of cold storage. Multivariate analyses coupled with metabolite annotation using databases such as Metlin and MassBank revealed putative identification of discriminant metabolites linked to different stages of cold storage of 'Kinnow' mandarin. In conclusion, LC-QTOF based metabolomics can be a powerful tool to unravel the mechanisms underlying the postharvest cold storage-induced metabolite changes ultimately leading to fruit quality.

P-144	Metabolomics approach to investigate food waste valorisation through fermentation

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Brewers' spent grain (BSG) is a nitrogen rich and fiber rich by-product generated in the beer manufacturing process, produced in high volumes throughout the world. Here we have investigated the use of biofermentation to add value to food waste materials, such as spent grain. In this study we use solid state fermentation (SSF) using GRAS state fungi for valorization of BSG to enhance its nutrient content. Subsequently, untargeted GC – MS based metabolomics was used to analyse the constituents at different stages of fermentation, followed by statistical analysis. Initially, we used various solvents to extract extracellular metabolites from spent grain, and found methanol to extract more metabolites, compared to water, hexane, dichloromethane and ethylacetate. Additionally, we investigated between alkylation and silylation for derivatization prior to GC – MS analysis, in which silylation provided better results. The metabolites attributing to significant changes between unfermented BSG and fermented BSG were determined using multivariate data analysis and searched against NIST mass spectral library for identification. We observed increased levels of amino acids, some fatty acids, vitamins and antioxidants in fermented BSG when compared to unfermented BSG. This data provided insight on the variations in the metabolites observed during fermentation, proving that biofermentation enhanced BSG's nutrient content. In conclusion, this study reveals the importance of a metabolimcs study to provide information to shed light on valorization in fermentation. Furthermore, data from metabolomics studies can be used to find novel applications for food wastes and innovative processing methods for valorization of waste materials.

P-145	Characterisation of the Human breastmilk using "multi-omics" analysis.

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Human breastmilk is considered as the most complete form of nutrition for infants. Composition of breastmilk can change in response to many maternal factors but also to match the infant's needs. However, the precise composition and evolution of metabolites, oligosaccharides and lipids is poorly documented. In the present study, we proposed the first detailed multi-omic description of breastmilk. We developed methods to analyse the metabolites, oligosaccharides and lipids contained in breastmilk by combining optimized extraction procedures and liquid chromatography coupled to high resolution mass spectrometry. All the developments and analytical validations have been made with a commercial pool of colostrum. Then we analyzed the global composition of breastmilk samples collected at days 2, 3, 4, 5 and 6 post-partum (pool of n= 13 to 124 / sampling date). Data treatment was made essentially using the Workflow4Metabolomic platform (http://workflow4metabolomics.org) while multivariate and univariate analyses were then performed to highlight relevant metabolic differences. Using these combined approaches, we were able to detect and annotate more than 200 metabolites, 80 oligosaccharides and 500 lipids in breastmilk. The dynamic analyses of these compounds at different days of lactation evidenced differences in terms of concentrations between day 2 and day 6. Developed and validated methods will be used to further study individual samples from mother-child cohorts, allowing to analyze the impact of various maternal and environmental factors on the composition of milk, and their impact on infant growth and health.

P-146	RANCIDITY IN ANCIENT GRAINS; the solution from Metabolomics

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Now a days the consumption of whole grains has increased due to the health benefits associated with them. There is much already reported about these health benefits but not much about the issues surrounding their use in food processing. What is missing, is a deep understanding of how one of the most valuable components -the lipids- become degraded and how can we stop that. Metabolomics is the technology we need to develop a deep understanding of the important molecules and what happens to them: starting from the fatty acid composition, then, tracking down until reaching the volatiles level. Profiling of the volatile molecules is our target since the major problem the food industry is facing is the short shelf-life of whole grain products due to their susceptibility to rancidity. Rancidity is the organoleptic perception of specific sub-products from lipid oxidation being firstly detected by the consumers for their characteristic smell. Using GCxGC-MS together with other analytical instruments, we have done an intense study to find common volatiles that we could target as an indicator of rancidity in a variety of whole grains including ancient grains like teff, quinoa and buckwheat and the traditional barley and sorghum. We can relate those volatiles to the fatty acid composition and also with different storage conditions to provide a better knowledge of the process that leads to rancidity, and therefore new options for the food industry.

P-147	Chronic addition of blueberries to the diet modifies the urinary metabolite profile in humans as shown by a LC-HRMS-based metabolomic study

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Blueberry consumption is widely known to benefit human health and is supported by numerous scientific publications. Consuming berry fruits enhances both cognition and motor control in animals. Blueberries contain relatively high concentrations of phytochemicals including anthocyanins, procyanidins, and chlorogenic acid. Here we report the analysis of urinary metabolites following 6-month consumption of blueberries by elderly humans. Our hypothesis was that chronic consumption of a dietary-relevant amount of blueberries has a measureable effect human metabolism. We attempted to detect and identify human urinary metabolites that were 1) derived directly from blueberries and 2) endogenous human metabolites that were altered as a result of consuming blueberries. This study included a detailed assessment of cognitive function, not reported here. Using RPLC-ESI-HRMS, we detected 1946 molecular features (MF) in negative mode and 1846 MF in positive mode. Currently, statistical analysis has included principal component analysis and T-Test. Twenty five metabolites were detected that either increased or decreased with p-value<0.05 and a fold-change>2. Attempts to identify these metabolites chemically are on-going, but several have been identified as ferulic acid and valerolactone metabolites, which are probably derived from blueberries. Furthermore, metabolite data downloaded from the Human Metabolome Database was used with TASQ (Bruker Daltonics) quantitation software to detect and semi-quantify 326 known urinary metabolites. We have established that a relatively small amount of blueberry added to the diet produces a measureable change in urinary metabolite profiles. As yet we have not identified any endogenous human metabolite that is altered as a result of blueberry consumption.

P-148	Metabolic profiling study of food samples – determination of saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), sterol, prenol and esters by using DI-SPME-GCMS

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The currently used GCMS based metabolites profiling methods for analysis of fatty acids in food samples have an obvious disadvantage, such as interfered by their derivatives in the analytical samples. Therefore, the obtained levels of fatty acids results are total free fatty acid derivatives. However, a technology of direct immersion solid phase microextraction technology coupled to a gas chromatography-mass spectrometry platform (DI-SPME-GC-MS) can significantly avoid the interference between fatty acids and others. This method can also offer for simultaneous determination of saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), fatty acids esters, sterol and prenol lipid species in wheat, barley and canola. The metabolites are extracted by Methanol: Acetonitrile: dH2O (2:2:1 v/v) solvent and followed by DI-SPME sampling & injection technology. The saturated fatty acids (SFAs), unsaturated fatty acids (UFAs), sterol lipids, prenol lipids and esters are extracted and analysed with high selectivity and sensitivity in comparison with syringe injection. It is no doubt, the DI-SPME-GC-MS method can be a practical method for monitoring saturated fatty acids (SFAs), unsaturated fatty acids (UFAs) and esters diversity between different food samples which include post-harvest stored grain and oil seeds. This method could be a validate method to be used for analysis levels of sterol and other lipid components in food sample for human daily intake.

P-149	Mapping and Chemometric Pattern Analysis on the Basis of Flavonoid DB in Korean Agro-food Resources

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Flavonoids are the most important group in secondary plant metabolites and generally produced as defense mechanisms against pathogens and disease organisms. These compounds have been reported to play an important role in the preventive aspects in human disease. In this study, the flavonoid chemometrics and maps was constructed using 3,205 quantitative values based on the already established flavonoid database from a total of 268 Korean agro-food resources. The UPLC-DAD-QTOF/MS based flavonoid profiles can be visualized as chemometrics through alignment and standardization procedures. Furthemore, the chemometrics can be variously mapped through clustering by PLS-DA modeling (SIMCA-P 11.0 software). In particular, since the teas(green and black) and pulses(soybean, kudzu vine) contains high levels of flavanol and isoflavone, respectively, clusters were formed independently from other groups such as vegetables, fruits and cereals in the food group map. This whole map can be further subdivided and enlarged for each food group. As a new research area in metabolomics, the metabolites mapping using complicated data matrix, could be provided and predicted numerous results including useful informations and hypotheses about scientific phenomenon hard to understand in specific classes as wells as used like an actual map. In future, these research could be applicated that the metabolic map constructed from metabolites associated with metabolic fluids of animal and human, and found easily therapeutic drugs.

P-150	NMR metabolomics analysis of urine from elderly men fed a high protein diet

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Higher protein diets have been promoted as beneficial for maintaining muscle mass in the elderly. However, the metabolic consequences of such diets are poorly understood. This study used 1H Nuclear Magnetic Resonance (NMR) spectroscopy to evaluate the urinary metabolite profiles of elderly men following a high protein diet compared to an adequate protein diet. For this study, 30 healthy men (74.2 ± 3.6 years) were randomised to consume either a high protein (1.6 g protein/kg body weight/day) or an adequate protein diet (0.8 g protein/kg body weight/day) for 10 weeks. Urine samples (24-hour collection) were collected before and after the intervention. These were analysed for creatinine, urea, and uric acid by colorimetric assays. Untargeted metabolomics was performed using a 700 MHz NMR spectrometer. Metabolites were identified using 2D NMR techniques and quantified using Chenomx software. Data were analysed using univariate and multivariate statistics. Preliminary results are available for nitrogenous compounds. Total nitrogen excretion was greater after the high protein diet at 15.3 ± 0.5 g N/day vs. 7.1 ± 0.5 g N/day (interaction time and diet, p<0.001). Urinary metabolites related to nitrogen excretion were increased after altered protein intake, suggestive of additional metabolomics changes that will be elucidated with the untargeted NMR results.

P-151	Authentication of two different fish oils by qualitative lipid profiling using semi-targeted approach on QTRAP platforms

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Globally, there is increasing consumer awareness on traceability and authenticity of fish and fisheries products. Fish oil, rich in the health promoting poly unsaturated fatty acids (PUFA) is in high demand in health food markets. The price and nutritional value of fish oils are determined by factors such as fish species used, processing method and storage. The differences in price between such oils may lead to mislabelling and adulteration. In this work, we demonstrate a proof of concept that qualitative shotgun lipid profiling using mass spectrometry can be used to differentiate between two different fish oils. Five samples each of Sardine (Sardinella longiceps) oil and Shark (Echinorhinus brucus) liver oil were diluted and directly infused in a quadrupole-linear ion trap SCIEX QTRAP® 4000 system. Data was acquired for 23 precursor ion scan and 4 neutral loss scan experiments in positive ionization mode. Similarly, in negative ionization mode data was acquired for 52 precursor ion scan and 5 neutral loss scan experiments. The lipid identification was performed using the LipidView™ Software for lipids present in the fish oil sample. Multiplexed precursor ion scan and neutral loss scan could identify many lipid classes based upon the precursor ion and neutral loss information. Cholesteryl ester, Diacyl glycerol, Triacylglycerol, Monoalkyldiacylglycerol, Phophatydyl choline and Sphingomyelin were the major lipid class identified. Statistical analysis including principal component analysis and student t-test done using MarkerView™ software could clearly distinguish between the sardine oil and shark liver oil.

P-152	Geographical Origin Discrimination of Oolong Tea (TieGuanYin, Camellia sinensis (L.) O. Kuntze) Using Proton Nuclear Magnetic Resonance Spectroscopy and Near-Infrared Spectroscopy

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A total of 90 Oolong tea samples were collected from three different growing places in Fujian province of China. Both proton nuclear magnetic resonance (1H NMR) and near-infrared spectroscopy (NIR) were used to analyze the collected tea samples. With the aid of chemometrics methods, differential components in 1H NMR data and characteristic wavenumbers from NIR spectra were identified. Since NMR and NIR provide complementary information for tea samples, data fusion was carried out by combining 1H NMR and NIR spectra of the collected tea sample. Experimental results showed that a better discrimination accuracy of geographical origins of Oolong tea could be achieved by combining NMR and NIR data (86.2-95.8%), as compared to using NMR data (68.2-78.7%) or NIR data (80.0-89.3%) alone. The current data suggested that a combination of NMR and NIR methods could serve as an efficient way for geographical origins discrimination and qualitative control of Oolong tea.

P-153	Differentiation of pork meat quality using GC-TOF-MS-based metabolite profiling

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The pork meat quality is greatly affected by many factors, including pig varieties, pork production areas, and cooking methods. Pork quality is conventionally verified by sensory evaluation and compositional analysis but there are remaining challenges on its result reproducibility and correlation between them. Therefore, comprehensive profiling on taste- and flavor-related metabolites in pork meat is required to clarify its potential discriminatory attributes of quality. Pork meat obtained from different origins (Japan, America, and Canada) was chosen as representatives to evaluate each meat quality. Raw- and roasted-forms of the meat was analyzed using GC-TOF-MS to detect primary metabolites. The acquisition data matrix was visualized by principal component analysis (PCA), and contribution of each metabolite was obtained from resulting PCA loading plot. One hundred peaks were detected wherein 60 of them were annotated. PCA score plot clearly indicated that the first two principal components (PC1 and PC2) were able to discriminate pork meat according to their origins and cooking state (raw or roasted), respectively. The metabolite profiles of Japanese pork in both raw- and roasted-forms were found to be distinct according to each form. On the other hand, the profiles of American and Canadian pork were gathered together despite their different forms. Moreover, the PCA plot revealed discriminant metabolites that are thought to be important to pork quality, such as sweetness-related amino acids (serine and glycine), umami-related amino acid (glutamic acid), and nucleic acid-related compound (inosine). Thus, GC-TOF-MS-based metabolite profiling has great potential to select metabolite index which discriminate pork quality.

P-154	Mapping of Greek olive oil using FT-ICR mass spectrometry flow injection analysis and multivariate data analysis

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Olive oil consumption is a product of high economic importance, and there is an urgent need for quality and authenticity control. Numerous analytical methods including HPLC-UV, GC-MS, LC-MS, and NMR have already been employed. In this study, we used magnet resonance mass spectrometry (MRMS) using flow injection analysis (FIA) and multivariate data analysis (MDA) to differentiate Greek olive oils from different regions. Multiple parameters influencing olive oil composition were investigated. 300 samples were collected over two years covering the olive oil producing areas of Greece. The oil was dissolved and diluted in a CH2Cl2/MeOH + 10 mM ammonium acetate solution. For each sample, five replicates were measured on a solarix XR 7T (Bruker Daltonik) using a 100µl full loop FIA in electrospray ionization negative ion mode. Single MS spectra were acquired in 2.5 minutes. Due to the ultra-high mass resolution of MRMS, olive oil can be analyzed without chromatographic separation. Mass peaks were deisotoped, combined into features, and aligned across samples. The evaluation was done with PCA using MetaboScape (Bruker Daltonik), and with OPLS-DA in SIMCA-P (Umetrix). Tentative annotation of metabolites via database matching took into account accurate mass and isotopic fine structure information. Samples could be clustered according to geographical origin using OPLS-DA methods, while olive tree variety appear less important. PCA loading plots revealed the metabolites responsible for samples classification, most important are Oleic and linoleic acids. Overall, the FIA MRMS metabolic profiling workflow is a fast and reliable approach for olive oil quality and authenticity control.

P-155	Effects of dietary Allium hookeri on growth performance and anti-oxidant activity in young broiler chickens

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CO-AUTHORS:

The aim of this study was to evaluate the effect of dietary supplementation of Allium hookeri root on anti-oxidant activities and growth performance in young broiler chickens. A total of 125 male Ross-708 broiler chickens (n = 25 birds/group) were fed standard diets supplemented with root or fermented root of A. hookeri at two different levels (1% or 5%) for 3 weeks from hatching. Control birds were provided with non-supplemented basal diets. Body weights, feed conversion ratios (FCRs), heme oxygenase (HMOX), aflatoxin B1 aldehyde reductase (AFAR), superoxide dismutase 1 (SOD1), catalase (CAT) enzyme, and malondialdehyde (MDA) were measured levels were measured in 21-day-old birds. The results showed greater body weight gains at day 14 and FCRs at day 21 in chickens fed diets supplemented with 1% A. hookeri root, as compared to the control group (p < 0.05). Up-regulated transcript levels of AFAR, Hmox1, and CAT were observed in the jejunum of chickens fed diets supplemented with A. hookeri. The serum levels SOD and CAT were significantly increased (p < 0.05) in groups treated with A. hookeri, whereas MDA levels were decreased in groups fed diets supplemented with A. hookeri, as compared to the control group. These results indicated that an optimum level of dietary A. hookeri as a feed additive to young broiler chickens influences growth and improves anti-oxidant activities.

P-156	Discrimination of Origin of Soybeans by Volatile Metabolites

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Soybean (Glycine max) is one of the crops cultivated globally in various areas and generally consumed in many Asian countries. Volatile metabolites of soybeans are significantly affected by climate and soils, where they are cultivated, as well as variety. Thus, volatile metabolites can be one of important factors for the determination of origins of soybeans. In this study, various pretreatment procedures were performed prior to the extraction of volatile metabolites to prevent any chemical and biochemical changes, in particular, in lipoxygenase activity during sample preparation. Then, volatile metabolites were extracted using solid-phase micro extraction (SPME) and analyzed by gas chromatography-mass spectrometry (GC-MS). All the datasets obtained were processed by multivariate data analyses such as principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) to determine the key volatile metabolites related to the discrimination of their origins.

P-157	Comparative Profiling for Authentication of Deep-fired Oils by Using Gas Chromatography-mass Spectrometry

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A novel approach for authentication of deep fired oils by using gas chromatography mass spectrometry was developed. Untargeted profiling of oil samples combined with multivariate analysis was performed on the elucidation of deterioration process of edible oil, which suggested monoglycerides are the most contributive molecules to the discriminatory effects of fresh edible oil and deep fired oil. Furthermore, accumulation behavior of monoglycerides under continuous heating processes was observed. By using the quantitative results, the concentrations of monoglycerides in deep fired oil and gutter oil were determined. Besides, admixture adulteration of fresh edible oil adulterated with deep fired oil was distinguished at very low concentration (approximate 1%). This described method with unambiguous qualitative and quantitative information being elucidated, proposed six of monoglycerides as endogenous indexes for comprehensive assessment of the degradation state of cooking oil.

P-158	Metabolic profiling of antioxidant supplement MP using plasma 1H NMR-based metabolomics in humans

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Aging and age-related diseases in humans are mainly caused by oxidative stress through over-production of reactive oxygen species (ROS). In our previous study, multi-micronutrient supplement containing antioxidant nutrients and phytochemicals (MP) resulted in higher antioxidative activity by increasing serum folate with resistance to DNA damage and LDL oxidation to subjects. In this study, plasma samples were analyzed using 1H NMR. The metabolic profiles before MP supplementation and after 8-week MP supplementation were clearly separated based on multivariant analyses of principal component analysis (PCA) and orthogonal partial least square discriminant analysis (OPLS-DA). Twenty-seven metabolites from variable importance plots were screened before MP supplementation and after 8-week MP supplementation. The 27 metabolites had significantly increased levels of betaine but significantly decreased levels of choline, serine, and threonine after MP supplementation. These results suggested that MP supplementation could activate folate cycle to increase resistance to DNA damages.

P-159	Effects of dietary taurine supplementation on metabolomic variation in grouper (Epinephelus coioides) intestine

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In this study, 1H NMR-based metabolomics approaches in combination with multivariate pattern recognition technologies were applied to investigate the impact of dietary taurine on intestine metabolomic variations of grouper (Epinephelus coioides). Fish was fed by one of four diets containing 0.0, 0.5, 1.0 and 1.5% supplemental taurine for 84 days feeding period. A total of 25 metabolites were found to change significantly in intestine of fish fed with different taurine-added diets. These metabolite changes basically involved in the varied kinds of metabolic pathways including biosynthesis of biological substances, protein digestion and absorption, lipid metabolism and lipolysis, carbohydrate metabolism, digestion and absorption, and ATP-binding cassette transporters (ABC transporters). The substantial cores of all the metabolic pathways are exerting a regulation on fish body physiological state and suggesting that taurine supplementation in the diet would significantly affect the intestine metabolomic, improved glucose utilization and amino acid uptake, promoted the protein synthesis and further accelerate the fish growth performance. Moreover, this taurine benefit was supplementation level sensitive, where great metabolism change and maximum fish performance were at 1.0% supplemental taurine under this experimental condition. Our results demonstrate that the utility of NMR-based metabolomics method can help to find the proper content of taurine in the aquafeeds and provide new insights into taurine roles on aquaculture at metabolic responses.

P-160	Metabolite profiling of different parts between brown and white beech mushroom (Hypsizigus marmoreus)

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Beech mushroom (Hypsizygus marmoreus) is a well-known for its beneficial health effects such as anticancer, antiviral, and antihypertensive activities. Only few studies had been reported about those bio-active metabolites. In this study, comparative metabolite profiling of two parts (cap and strip) of brown and white beech mushrooms was performed by gas chromatography-time-of-flight-mass spectrometry (GC-TOF-MS) and ultrahigh performance liquid chromatography-linear trap quadrupole-ion trap (UHPLC-LTQ-IT) MS/MS with multivariate statistical analysis. The two parts were clearly separated by PC1 and two strains were separated by PC2 in principle component analysis (PCA) score plot. Heat map analyses revealed that levels of most amino acids and hypsiziprenols were high in cap than strip part in both strains. Those of metabolites also showed a high positive correlation with antioxidant activity. Whereas, fumaric acid, malic acid, fructose, and benzoic acid were relatively high in stipe part. Discriminated metabolites between brown and white strains such as valine, leucine, serine, threonine, hypsiziprenol AA family, hypsiziprenol A10, and hypsiziprenol A11 were dominantly found in brown strain. However, the levels of ornithine, tryptophan, succinic acid, citric acid, and myo-inositol were relatively high in white strain than brown strain.

P-161	Investigating the Effect of Saskatoon Berry Syrup Addition on the Antioxidant Properties and Phytochemical Content of Rooibos Tea (Aspalathus linearis)

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Saskatoon berry syrup (SBS) contains many vitamins, minerals and functional bioactive components that contribute to its desirable flavor, color and antioxidant properties. Thus, the addition of SBS to rooibos tea (RT), a popular caffeine-free beverage with a unique taste, could enhance the healthfulness of the product. The objective of this study was to examine the effect of SBS addition (10% w/w) on the antioxidant properties and phytochemical content of RT fortified with vitamin D3. Six formulations (RT, RT with SBS, RT with SBS and vitamin D3, RT with vitamin D3, green tea (GT), and GT with SBS) were evaluated for total antioxidant capacity (TAC), total anthocyanins and phytochemical content by integrating an untargeted foodomics approach. Results revealed that GT with SBS had significantly higher (P<0.05) TAC compared to other samples. No significant differences were observed between the TAC of GT, RT with SBS and RT with SBS and vitamin D3. RT with and without vitamin D3 had the lowest TAC and were comparable to a 10% diluted sample of SBS. No significant differences were observed in total anthocyanins between teas with added SBS. The addition of SBS contributed several polyphenols, particularly flavonoids to teas. A total of 9 and 10 phytochemicals were significantly enhanced in RT with SBS compared to RT alone and GT with SBS compared to GT alone, respectively, among which 6 phytochemicals were similar including pyrogallol. The addition of SBS could be a favorable natural additive that may provide additional health benefits including antioxidant properties to RT.

P-162	GC-MS based metabolomic studies of blueberry (Vaccinium spp.) and chokeberry (Aronia melanocarpa) with various geographical origins in Korea

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Berries have attracted attention as a health food with various effects on physiological activities. Recently, emerging knowledge is being gained on the mechanisms related to environmental factors that may affect chemical composition or metabolites in berries. We performed metabolite profiling of blueberry (Vaccinium spp.) and chokeberry (Aronia melanocarpa) derived from 7 different geographical origins in Korea using gas chromatography-time-of-flight-mass spectrometry and multivariate statistical analysis. According to principal component analysis (PCA) score plot, berries were separated not only by different species but also by regional groups such as latitude (35°N and 36°N region). These distribution showed a greater effect of geographical origins on the metabolites than the cultivar or harvest time. In accordance with pathway analysis, most amino acids levels were relatively high in chokeberry compared to blueberry, while blueberry contained high levels of most sugar derivatives. In both blueberry and chokeberry cultivated in the region of 35°N, levels of amino acids and organic acids, including valine, threonine, aspartic acid, pyroglutamic acid, GABA, glutamic acid, and lactic acid showed relatively higher than those of cultivated in the region of the region of 36°N. Whereas, content of sucrose was specifically high in berries cultivated in the region of 36°N than 35°N. In correlation analysis, most amino acids and organic acids showed a high positive correlation with duration of sunshine, while sugars and sugars alcohols showed positive correlation with rainfall. From these results, we proposed that the primary metabolism of berries was affected by geographical origins and climatic conditions.

P-163	Analysis of gas chromatography-mass spectrometry to identify urinary metabolites after increased intake of whole grain, vegetables, and fruits in healthy adolescents

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Dietary factors in plant-based diet are important for the prevention of chronic and metabolic diseases. Especially, phytonutrients in vegetables and fruits have been recognized as major contributors for the decreased level of oxidative stress. In this study, switching the dietary behaviors of Korean healthy adolescents to high consumption of vegetables and fruits was investigated using blood test of the plasma and untargeted metabolic profiling of the urine. Eighteen healthy adolescents were provided for 8 weeks with whole grain, vegetables, and fruits as the main food source. Anthropometric measurements, blood profiles, and dietary intake were measured and compared before and after the plant-based diet. After the plant-based diet, Weight and body mass index were significantly decreased. Also, biological antioxidant potential of the plasma was increased. The chromatogram data acquired by gas chromatography-mass spectrometry analysis was processed. From the orthogonal partial least squares-discriminant analysis model, a total of 835 metabolites that played important role in the separation between before and after the plant-based diet were selected according to the variable importance in projection value ?1.0. Seven endogenous metabolites were identified; glycine, hydrocinnamic acid, succinic acid, stearic acid, tartaric acid, trans-ferulic acid, and fumaric acid, which are known to have antioxidant potential and anti-tumorigenic effects, in addition, to reduce low density lipoprotein cholesterol. Overall, this dietary metabolomics approach will provide a better understanding of alterations in the endogenous metabolites affected by the plant-based diet and these alterations were correlated with change of anthropometric parameters and blood profiles.

P-164	Evaluation of carotenoids variations due to plant replicates for 50 pepper varieties using targeted metabolomics analysis platform

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Pepper (Capsicum spp.) is one of the most important fruit crop worldwide reaching more than 28 million metric tones harvested from the crop. Pepper is increasingly recognized as a good source of bioactive compounds that contribute many health benefits associated with hypertension, blood circulation, muscle pain, obesity, and age-related macular degeneration. The well-established bioactive compounds in pepper include capsaicinoids such as capsaicin and capsiate, and carotenoids. Capsaicinoids are responsible for the strong and hot taste of the fruits, also known as pungency. Although the key enzyme leading to the production of capsaicinoids is capsaicin synthase, little is known about a specific gene associated with production of a few capsaicin compounds. Carotenoids are an important group of pigments responsible for the fruit colors. Regarding genes encoding carotenoids biosynthesis enzyme, only a few genes has been reported to date. Recently, genome-wide association analysis with metabolites in crops varieties reported numbers of genes associated with corresponding metabolites. In order to obtain meaningful and real genes for the associated metabolites, care must be taken regarding sampling, sample extraction, and analytical platform. Particularly, metabolite variations due to plant replicates are one of most important considerations before performing genome-wide association analysis for metabolite trait. In this study, we developed a targeted metabolomics analysis platform to evaluate variations of several carotenoids due to plant replicates using 50 pepper varieties.

P-165	Metabolomic approach for biological active compounds in the Korean alcoholic beverage brewed with traditional nuruk

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CO-AUTHORS:

Nuruk, is a Korean traditional fermentation starter. The taste and flavor of Korean alcoholic beverage 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. In this study, we brewed Korean alcoholic beverage using 16 different nutritional ingredient-based traditional nuruk and then investigated ACE inhibitory effects and changes in metabolites. The metabolites of Korean alcoholic beverage were simultaneously analyzed by liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS). Metabolites profiling of Korean alcoholic beverage were affected by nutritional and microbial composition of fermentation starter. As a result, the samples taken at different Korean traditional nuruk were clearly distinguishable in the score plot generated by combining PC1 (33.11% of the total variance) with PC2 (16.39% of the total variance). To investigate the metabolites related to the biological active compounds of nuruk-based Korean alcoholic beverage, the components related to functionality were explored through the metabolite profiling, correlation analysis, and heatmap analysis. Acquired a list of candidate biological active compounds for Korean alcoholic beverage and discovered bioactive compounds that actually exist in the nuruk-based Korean alcoholic beverage. The Korean alcoholic beverage with a high ACE inhibitory effect showed significantly high contents of two metabolite ingredients (p<0.001). This study revealed that mass based metabolites profiling was useful in helping to understand the metabolite differences by nutritional and microbial composition of fermentation starter.

P-166	Meat and fish consumption lead to differences in plasma N-acetylaspartate and trimethylamine N-oxide concentrations in healthy men

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Introduction/Objectives: Nutritional biomarkers are required to objectively evaluate dietary intake. However, few studies were clarified the biomarker for meat and fish intake. In this study, we investigated whether there were differences in the profiles of postprandial plasma metabolites by meat or fish intake. Methods: In a randomized crossover study, 8 healthy men alternately consumed one of five test meals containing 100 g of beef, pork, chicken, mackerel and salmon on a different day. Fasting and postprandial plasma samples were analyzed using CE-TOFMS and multivariate data analysis. Results: OPLS-DA identified 34 metabolites that could distinguish between meat and fish intake. Among the metabolites, N-acetylaspartate, hydroxyproline and 5-hydroxylysine concentrations were significantly higher after meat intake than fish intake, and also trimethylamine N-oxide (TMAO), Val, hydroxyproline, creatine, Ile, Met, Tyr and methionine sulfoxide concentrations were significantly higher after fish intake than meat intake. Interestingly, high N-acetylaspartate concentrations were observed only after meat intake, but there was no difference between before and after the consumption of fish. Similarly, high TMAO concentrations were observed only after fish intake, but there was no difference between before and after meat intake. Receiver-operating characteristic curve analysis showed that N-acetylaspartate could predict meat intake with an AUC value of 0.9634, and also TMAO could predict fish intake with an AUC value of 0.9342. These results suggest that N-acetylaspartate and TMAO are good candidate biomarker to evaluate meat and fish intake, respectively.

P-167	Barley Seedling Extracts Inhibit RANKL-induced Differentiation, Fusion and Maturation of Osteoclasts in the Early-to-late Stages of Osteoclastogenesis

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The number of patients with osteoporosis is increasing in worldwide, and a decrease in bone mass is a major risk factor for fracture. The prevention of bone loss is critical for improving the quality of life for patients. However, the long-term use of anti-osteoporotic agents is limited due to their side effects. Barley has been traditionally ingested for thousands of years as a safe, natural food with pharmaceutical properties, and its seedling can enhance the biological activity of the medicinal components found in food. This study aimed to elucidate the anti-resorptive activity of barley seedling and its mode of action. Barley seedling extracts (BSE) dose-dependently inhibited RANKL-induced osteoclast differentiation with alteration of I?B degradation, c-Fos and NFATc1 molecules in the early-to-middle stages of osteoclastogenesis. In the late phase of osteoclastogenesis, BSE also prevented DC-STAMP and cathepsin K, which are required for cell fusion and bone resorption, such as osteoclast function. In conclusion, barley seedling from natural foods may provide long-term safety and be useful for the prevention or treatment of osteoclast-mediated bone metabolic diseases, including osteoporosis

P-285	Volatile metabolite screen for beer and wine using large volume headspace-GCMS analysis

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Volatile metabolites are important components of beer and wine, contributing to the aroma, flavour and quality of the finished product. Yeast-derived fermentation products, acetate- and ethyl-esters, and higher alcohols, together with monoterpenes from hops or grapes impart positive fruity, floral or less sought-after solvent like characters in beer and wine. The yeast strain employed for fermentation and the nutrient supplements used can directly affect the concentrations of these aroma compounds. In addition, the choice of hops is a crucial element for beer production that drives unique flavour attributes. A combined targeted/untargeted metabolomics approach using large volume headspace-GCMS was developed to monitor a wide range of volatile metabolites in fermented beverages. The method described in this poster accurately quantifies 18 fermentation products by SIDA and simultaneously performs untargeted analysis of a broad range of volatiles present in the headspace of beer or wine samples. The metabolite profiling data can be processed using tools such as XCMS and CAMERA and the untargeted analysis typically results in the detection of 30-50 metabolites in the headspace. Identification of metabolites is performed using in-house libraries containing mass spectra and retention index values obtained from pure reference standards.

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O-48	LC-MS-based metabolomics revealed SLC25A22 as an essential regulator in aspartate-derived amino acids and polyamines in KRAS-mutant colorectal cancer

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Colorectal cancer (CRC) is the third most common cancer causing death worldwide. SLC25A22, which encodes the mitochondrial glutamate transporter, is overexpressed in CRC and is essential for the proliferation of CRC cells harboring KRAS mutations. However, the metabolic effect of SLC25A22 on CRC cells has not been characterized on a metabolome-wide scale. In the study, global and targeted metabolomics based on ultra-high performance liquid chromatography (UHPLC) coupled to mass spectrometry (MS) were used to evaluate effects of SLC25A22 on metabolism in KRAS-mutated CRC cells. Global metabolomic analysis of KRAS-mutant DLD1 cells with or without SLC25A22 knockdown identified 35 differentially regulated metabolites, which were primarily involved in glutaminolysis, urea cycle and polyamine metabolism. Targeted metabolomic analysis of TCA cycle intermediates, non-essential amino acids, and polyamines revealed that TCA cycle intermediates (except ?-ketoglutarate), aspartate (Asp)-derived asparagine, alanine and ornithine (Orn)-derived polyamines were all strongly down-regulated in SLC25A22 knockdown cells. Moreover, targeted kinetic analysis was performed with [U-13C5]-glutamine as the isotope tracer. The 13C-labeled urea cycle metabolites were not detected in SLC25A22 knockdown cells, suggesting the urea cycle was not triggered due to decreased levels of Orn. On the other hand, most 13C-labeled Orn-derived polyamines were significantly decreased in SLC25A22 knockdown cells and medium. Exogenous addition of polyamines could partly restore cell proliferation in SLC25A22 knockdown cells, highlighting their potential as oncogenic metabolites downstream of SLC25A22-mediated glutamine metabolism. Collectively, SLC25A22 promoted synthesis of asp-derived

O-60	A non-targeted UHPLC-HRMS metabolomics pipeline for metabolite identification; application to cardiac remote ischemic preconditioning

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In recent years, the amount of investigations based on non-targeted metabolomics has increased, although often without thorough assessment of analytical strategies applied to acquire data. Following published guidelines for metabolomics experiments, we report a validated non-targeted metabolomics strategy with pipeline for unequivocal metabolites identification using the MSMLS™ molecule library. We achieved an in-house database containing accurate m/z values, retention times, isotopic patterns, full MS and MS/MS spectra. A UHPLC-HRMS Q-Exactive™ method was developed and experimental variations were determined within and between 3 experimental days. The extraction efficiency as well as the accuracy, precision, repeatability, and linearity of the method were assessed, the method demonstrating good performances. The methodology was further blindly applied to plasma from Remote Ischemic Pre-Conditioning (RIPC) rats. Samples, previously analyzed by targeted metabolomics using completely different protocol, analytical strategy and platform, were submitted to our analytical pipeline. A combination of multivariate and univariate statistical analyses was employed. Selection of putative biomarkers from OPLS-DA model and S-plot was combined to jack-knife confidence intervals, metabolites VIP values and univariate statistics. Only variables with strong model contribution and highly statistical reliability were selected as discriminated metabolites. Three biomarkers identified by the previous targeted metabolomics study were found in the current work, in addition to three novel metabolites, emphasizing the efficiency of the current methodology and its ability to identify new biomarkers of clinical interest, in a single sequence. The biomarkers were identified to level 1 according to the

O-63	Investigation of drug-induced steatosis in HepaRG cells using untargeted LC-MS metabolomics

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CO-AUTHORS: *Leen Claes, Charlie Beirnaert, Robim Rodrigues, Kris Laukens, Tamara Vanhaecke, Adrian Covaci*

Background: Accumulation of lipids in the liver (steatosis) is a frequent Drug-Induced Liver Injury. It is the first stage of Non-Alcoholic Fatty Liver Disease (NAFLD) and can progress to non-alcoholic steatohepatitis, fibrosis, cirrhosis and carcinomas, resulting in liver failure and death. Early detection and prevention of NAFLD are crucial in toxicological research and healthcare. Here, a hepatic in vitro system combined with metabolomics was used to gain more information on the mechanisms of action of sodium valproate, a reference steatogenic drug. Experimental outline: We optimised a LC-MS based metabolomics platform and exposed HepaRG cell cultures to sodium valproate at two different concentrations (IC10 and a 1/10 dilution of the IC10) and two different time points (24 h and 72 h). The intracellular metabolites were recovered in a polar and non-polar fraction using liquid-liquid extraction, both extracts were analysed with optimised untargeted LC-MS platforms. After quality control, the data were subjected to PCA and PLS-DA to select features of interest. Results: Exposure of HepaRG cultures to sub-cytotoxic levels of sodium valproate invokes a clear alteration in the metabolome. Significant differences were observed that relate to adaptive mechanisms which eventually progress to lipid accumulation and steatosis. The metabolic changes include a conversion from lower weight to higher weight lipids and alterations in the levels of polar organic acids. Conclusions: Using untargeted LC-MS metabolomics on HepaRG cultures, a sodium valproate-induced intermediary adaptation of the metabolome is observed, progressing to steatosis.

O-80	Spatially resolved profiling of Polymyxin B induced acute kidney injury

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The global problem of advancing antimicrobial resistance let to a renewed interest in Polymyxin antibiotics. These antibiotics are commonly used as a last resort in cases of uncontrolled infection caused by gram-negative bacteria. Polymyxin efficacy is linked to known neuro- and nephrotoxicity in the clinic. In the current study we applied desorption electrospray ionisation (DESI) and matrix assisted laser desorption ionization (MALDI) mass spectrometry imaging (MSI) to investigate polymyxin induced changes in the tissue metabolome of rat kidney sections. Kidneys from animals receiving a high dose of Polymyxin B1 showed initial increased formation of inflammatory markers, e.g. diacylglycerols, eicosanoids, lysophosphatidylcholines. The formation of these markers was dose dependant (reduced after receiving a low dose of Polymyxin B1 or after a recovery period of 24 days). Consistently, kidneys from animals treated with Polymyxin B nonapeptide, which is known to be less nephrotoxic, showed reduced formation of the inflammatory markers. The localization of the markers was found to be in the renal cortex which is consistent with urinary biomarkers and histopathology findings locating the nephrotoxicity in the proximal tubules and to a lesser extent in the distal tubules. Applying multimodal MSI, we demonstrated the ability to correlate the location of a toxic compound within a tissue with the effects it caused. In the case of the presented study and toxicology studies in general, this correlation can be used to help understand the mechanisms responsible for the toxicity and help to reveal the underlying pathways.

O-82	Analysis of the metabolomic responses to high protein meals in women at increased metabolic disease risk

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Metabolic syndrome (MetS) is a cluster of risk factors for diabetes and cardiovascular disease. Recently, metabolomics has been applied to elucidate the key risk factors and changes associated with the MetS phenotype. This study aimed to comprehensively analyse the metabolic trajectories differentiating the responses of MetS from healthy women to high protein meals that included either low or high glycaemic index (GI) carbohydrates. Post-menopausal women (20 MetS, 20 healthy) consumed test meals on two separate mornings; the meals were high protein (30g whey), containing either high or low GI carbohydrates. Blood samples were collected at fasting and four postprandial time points. Metabolomic analysis were performed by applying a global metabolomics approach (LC-MS) accompanied by a UPLC targeted approach of amino acids. Dynamic probabilistic principal component analysis (DPPCA) was employed for dimension reduction, conforming with the correlation structure due to repeated measures. Linear mixed models (LMM's) were fitted, controlling by random-subject factors, to identify differentially regulated metabolites. A subset of metabolites, mainly amino acids, demonstrated significant differences over time between MetS and healthy women. A global metabolomic approach reveals differences in baseline metabolic profile between healthy and MetS women; furthermore, the postprandial trajectories of these metabolites differed. This suggests alterations in amino acid and carbohydrate associated metabolic networks. The identification of compounds which change in response to food will provide insights into metabolic differences between healthy and MetS women and will further inform nutritional strategies to reduce disease risk.

O-83	Faecal and serum metabolomics in paediatric inflammatory bowel disease.

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CO-AUTHORS: *Kaija-Leena Kolho, Alberto Pessia, Tytti Jaakkola, Willem de Vos*

BACKGROUND: Inflammatory bowel disease [IBD] is considered to result from the interplay between host and intestinal microbiota but its pathogenesis is incompletely understood. While IBD in adults has shown to be associated with marked changes in body fluid metabolomics, there are only few studies in children. Hence, this prospective study addressed the faecal and serum metabolomics in newly diagnosed paediatric IBD. METHODS: Paediatric patients with IBD undergoing diagnostic endoscopies and controls also with endoscopy but no signs of inflammation provided blood and stool samples in a tertiary care hospital. Blood inflammatory markers and faecal calprotectin levels were determined. The serum and faecal metabolomics were determined using ultra-high pressure liquid chromatography coupled to a mass spectrometer. RESULTS: Serum and faecal metabolite profiles in newly diagnosed paediatric IBD patients were different from healthy controls and categorized Crohn's disease and ulcerative colitis [UC] patients into separate groups. In serum, amino acid metabolism, folate biosynthesis and signalling pathways were perturbed in Crohn's disease; in UC also sphingolipid metabolic pathways were perturbed when compared to controls. In faecal samples, there was an increased level of several metabolites in UC in contrast to low or intermediate levels in Crohn's disease. There was a clear correlation with the level of inflammation, i.e. faecal calprotectin levels and the profile of various biologically important metabolites [carnosine, ribose and, most significantly, choline]. CONCLUSION: Characterization of inflammatory pattern using metabolomics analysis is a promising tool for better understanding disease pathogenesis of paediatric IBD. REFERENCE: J Crohn's and Colitis (2017). 11(3):321-334. PMID:27609529.

O-129	Spatial Imaging of Invasive ductal carcinoma specimens by Desorption Electrospray Ionisation Mass Spectrometry

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CO-AUTHORS: *Edward John, Francesca Rosini, Anna Mroz, James McKenzie, Zoltan Takats*

Breast cancer is a highly heterogeneous disease and one of the most prevalent form of cancers in women worldwide. Estrogen receptor (ER) measurement is a routine procedure for clinical management of breast cancer. The result from the test alone is used to determine which patients would benefit from tamoxifen. DESI (desorption electrospray ionisation) is a novel ambient ionisation technique that coupled with mass spectrometry imaging (MSI) has great potential in breast cancer diagnostics. Therefore, breast samples from 80 patients were analysed by DESI-MSI to understand the lipidomic differences between histologically normal and different molecular subgroups of breast cancer. The results from the negative ion mode dataset showed that DESI-MSI can be effectively used for discriminating between the malignant and histologically normal glandular tissues (with 95% accuracy), histological grades of tumour (with 72% accuracy) and ER status (70% accuracy). Lipid species belonging to phosphatidylethanolamine and phosphatidylinositol class were found to be in higher abundance in the ER-positive samples compared to ER-negative samples and morphologically normal glandular tissues. Additionally, 101 breast samples were analysed with REIMS (rapid evaporative ionisation mass spectrometry) and the spectral content generated was studied. Lipids belonging to the phosphatidylethanolamine class were found to give the highest contribution to the separation of normal and malignant tissue classes in REIMS and DESI-MSI dataset. As a diagnosis tool, DESI-MSI would allow cancer diagnosis to be more precise and more accurate compared to routinely used methodologies in histopathology laboratories as it is solely based on sample's biochemical features.

O-134	Metabonomic Study of Schizophrenia Using High-Resolution NMR Spectroscopy

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The mental disorder schizophrenia affects the way of thinking, feeling and acting and can result in a severely limited way of living for both the affected person and their carers. Causes and triggers of schizophrenia, a potentially multicausal disorder, resulting in imbalanced metabolite concentrations in the brain that can also transfer to associated body fluids, are still unclear. Therefore, we analysed blood plasma samples as a readily available body fluid in order to contribute to the understanding of the mechanisms involved in the pathogenesis and treatment of schizophrenia. In the present study we measured and analysed 130 blood plasma samples from subjects with first episode psychosis and a healthy control group using one and two dimensional high-resolution NMR spectroscopy. The patient samples were derived from new onset as well as from chronic patients, and samples before and after treatment with atypical antipsychotic medication (mainly quetiapine) with or without ethyl-eicosapentanoic acid augmentation were analysed. For data analysis statistical techniques such as principal component analysis and partial least squares analysis-discriminant analysis were employed to determine metabolite differences between the groups, in order to subsequently correlate them with associated metabolic pathways. We identified different lipid profiles between healthy and diseased people suggesting that altered phospholipid profiles, arising from reduced uptake or increased phospholipid breakdown, are potentially associated with the onset or course of the disease. Metabolite changes during treatment could also be observed. Our results add to the understanding of metabolite changes in the blood occurring in early onset schizophrenia.

O-146	Metabolomic application of the Intelligent Knife (iKnife): Rapid Evaporative Ionisation Tandem Mass Spectrometry (REIMS/MS) for In-Vivo Metabolite and Tissue Identification in the Case of Breast Cancer

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The intelligent knife (iKnife) uses aerosol formed on standard electrosurgical dissection which is aspirated into a time of flight mass spectrometer (TOF MS) equipped with a REIMS (rapid evaporative ionisation mass spectrometry) interface to acquire tissue specific MS signal. Although spectral fingerprint-based identification has been demonstrated to provide excellent tissue identification performance [1], mass spectra contain more information: details of the metabolites within the tissue. Normal and cancerous breast tissue samples were analysed ex-vivo using the iKnife system. Univariate analysis revealed significant marker ions between the tested tissue types then these ions were selected for MS/MS analysis. MS/MS spectra and exact mass of the ions showed that spectra mainly contained phospholipids and triacylglycerides. Fragment ion spectra of the selected significant ions revealed the presence of isomeric lipids. E.g. ion at 768.6 m/z provided characteristic fragments for PC(18:1/18:2), PC(16:0/20:3), PE(20:2/18:1) and PE(18:0/20:3) lipids. In order to investigate which lipids are responsible for correct tissue classification, multiple MS/MS spectra were acquired from normal and cancerous tissues. Fragment ions showed clear separation between the tissue types even when single ion MS/MS spectra were examined. These identified, significant lipids were used to compare healthy and cancerous tissues for better understanding of lipid metabolism and its alteration. iKnife spectra could reconstruct the lipid metabolism of breast cancer and cancer specific metabolic pathways were found. These ex-vivo and also in-vivo pathway analyses could improve diagnosis accuracy and could also be target of new treatments. [1] Angew Chem Int Ed Engl, 2009. 48(44): p. 8240-2.

O-154	Multi-omics Analysis of Esophageal Adenocarcinoma: How Lipid Metabolism Affects Cancer Progression

PRESENTING AUTHOR: *Jeffrey Molendijk, The University of Queensland Diamantina Institute, The University of Queensland, Brisbane, QLD, Australia, Australia*

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The incidence of esophageal adenocarcinoma (EAC) has increased in recent years, whilst the 5-year survival rate remains low at ~15%. EAC is associated with altered lipid metabolism, with obesity as a major risk factor. Conversely, cholesterol-lowering statin drugs are protective and attenuate growth and malignant potential of EAC cells. MALDI and spectroscopy studies report changes in the lipid profiles of EAC tissue compared to healthy squamous epithelium. Lipids affect cancer behavior by storing energy as well as regulating cell survival and apoptosis. However, the specific lipid metabolism pathways involved in the progression from the pre-cancerous condition called Barrett's esophagus (BE) to EAC remain unclear. To study these pathways, we conducted mass spectrometry (MS) -based proteomics and lipidomics experiments on 7 cell lines representing non-dysplastic BE, high-grade dysplastic BE and EAC. Lipid and proteins were extracted from cell pellets using a biphasic TBME/MeOH method. Proteomic profiling was performed on a QE+ MS (Thermo). Untargeted discovery lipidomics experiments were performed on a 1290 Infinity II/6550 Q-TOF LC/MS system (Agilent) whilst targeted MRM experiments were performed on a 6490 Triple Quadrupole MS system. Combined analysis of the proteomic and lipidomic changes were performed to identify lipid metabolic pathways differentially expressed in EAC progression. Our results show that lipids involved in energy storage and regulation of cell survival are associated with disease progression. Future work will verify candidate molecules in patient samples.

O-166	Urinary metabolic profiles associated with 5-year changes in biomarkers of glucose homeostasis: an NMR spectroscopy based study

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CO-AUTHORS: *Tea Skaaby, Maik Pietzner, Betina Thuesen, Matthias Nauck, Allan Linneberg*

As known for the last decades, type 2 diabetes mellitus (T2DM) represents one of the major health problems which will become more and more important due to the steadily increasing prevalence of overweight and obesity. During the last decade metabolomics studies were used to gain deeper insight into the pathogenesis of diabetes mellitus with promising findings. However, longitudinal metabolomics studies of possible subclinical states of disturbed glucose metabolism are limited. Therefore, the aim of this study was to analyze the associations between baseline urinary metabolites and 5-year changes in markers of glucose homeostasis using urine metabolites among 3986 participants of the population-based Inter99 study analyzed by 1H-NMR spectroscopy. The analyses revealed that several urinary metabolites, including alanine, betaine, 1-methylnicotinamide, trimethylamine and trigonelline, were found to associate with detrimental longitudinal changes in biomarkers of glucose homeostasis including fasting glucose, HbA1c and homeostatic model assessment for insulin resistance (HOMA-IR) index. For example, higher baseline levels of urinary alanine, betaine, N,N-dimethylglycine, creatinine and trimethylamine were associated with an increase in HbA1c from baseline to 5-year follow-up. In contrast, formic acid and trigonelline levels were associated with a decrease in HbA1c over time. The identified metabolites point to mechanisms within betaine and coffee metabolism as well as possible effects of the microbiome. Such knowledge may provide clues of pathogenetic mechanisms, targets for interventions, and might improve risk stratification of the population based on a readily obtainable bio fluid for clinical routine.

O-168	A combined metabolomics and lipidomics approach enables the stratification of acute-on-chronic liver failure patients according to their severity

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Acute-on-Chronic Liver Failure (ACLF) is a recently recognized syndrome characterized by acute decompensation (AD) of cirrhosis, an organ/system failure(s) and extremely poor survival. ACLF can be triggered by a precipitating event (e.g. bacterial infection) and is invariably associated with exacerbated systemic inflammation. According to the European Foundation for the study of Chronic Liver Failure (EF-CLIF), patients with ACLF can be classified into three groups, essentially according to the number of impaired organs. In the present project, we investigated whether metabolomics and lipidomics can identify potential new diagnostic biomarkers of ACLF. In our study, a cohort of 89 serum samples from decompensated cirrhotic patients with and without ACLF were analyzed and compared to healthy subjects by liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS). Two complementary methods involving both HILIC and C18 columns for metabolomics and C8 column for lipidomics were used in both positive and negative ionization modes. Data mining procedures using multivariate and univariate analyses were then performed to highlight discriminant metabolites. Our data confirmed the metabolic and lipidomic cirrhosis signatures obtained in previous studies, especially regarding to the levels of glycerophosphatidylcholines, amino acids and energy metabolites. Furthermore, our approach enabled to discriminate between decompensated cirrhotic patients with ACLF and those without ACLF, and a specific metabolite signature associated with the ACLF grade has been obtained. Taken together, our findings indicate that metabolomic and lipidomic profiling using LC-HRMS may contribute to describe the ACLF development in cirrhotic patients and provide new insights into the metabolomics changes detected.

O-169	Correlation of a plasma metabolite score with duration of hypoxia: a piglet study

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Hypoxic-ischemic encephalopathy (HIE) secondary to perinatal asphyxia is a leading cause of mortality and long-term neurologic co-morbidities in the term neonate. Moderate whole body hypothermia initiated within 6 hours from birth is the most successful intervention for treatment of moderate to severe HIE. This work strives for an early metabolic biomarker score for assessing the risk of developing moderate to severe HIE in order to provide an objective measure for aiding clinical decisions. From a consolidated piglet model for neonatal hypoxia-ischemia, plasma samples were withdrawn before and at different time points after a hypoxic insult. A set of three metabolites (choline, 6,8-dihydroxypurine and hypoxanthine) showing maximum correlation with hypoxia time was identified from an untargeted liquid chromatography coupled to tandem mass spectrometry experiment and a multivariate Partial Least Squares metabolite score for predicting hypoxia time was established. Its performance as a biomarker for perinatal hypoxia was compared to lactate which is currently considered as the gold standard. The metabolite score performed similar to lactate for plasma samples withdrawn before and directly after a hypoxic insult, both providing sensitivity and specificity values equal to 1. However, in samples collected 2 h after resuscitation, lactate levels were not suitable for identifying asphyxiated piglets, while the metabolite score provided enhanced predictive capacity. Results evidenced the usefulness of the metabolite score for an early assessment of the severity of hypoxic insults. Ongoing studies are testing its capacity for clinical diagnosis and patient stratification in multicenter trials involving newborns with HIE.

O-172	Ion mobility–mass spectrometry based high-throughput metabolomics facilitates metabolite biomarker discovery in colorectal cancer

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Colorectal cancer (CRC) is one of the most common and lethal cancers around the world. Untargeted metabolomics has been proved as a powerful technology to discover metabolite biomarkers for CRC diagnosis. Here, we first developed an ion mobility–mass spectrometry (IM-MS) based high-throughput method for large-scale untargeted metabolomics. The analysis time for each sample is optimized as short as 3 minutes. We analyzed 194 tissue samples from CRC patients within 18 hours. The results showed that more than 90% of metabolites in quality control (QC) samples have RSDs less than 30%, proving the excellent stability and reproducibility of the method. For each sample, roughly 1000-1200 metabolites were detected in the 3-min analysis, and 300-400 metabolites were identified. Next, 80% of samples were randomly selected as training data, and the rest were used as validation data. In training data, cancer tissue and para-cancer tissue samples of CRC patients can be clearly differentiated using orthogonal partial least squares discriminant analysis (OPLS-DA). 125 dysregulated metabolites were selected with statistical tests of OPLS-DA (VIP > 1), fold change (FC > 1.3), paired t-test (p < 0.05). The combination of 12 potential biomarkers out of 125 metabolites was further optimized using LASSO regression. The AUC value in validation data was 0.98, with sensitivity of 94.0% and specificity of 100%. We will further validate the biomarker in 800 plasma samples from CRC patients. In conclusion, our IM-MS based high-throughput metabolomics can effectively facilitate biomarker discovery, and has the great potential for large-scale metabolomics.

O-179	Urinary metabolomics-based sub-phenotyping of a large-scale multi-omics asthma cohort identified tryptophan dysregulation associated with asthma

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Aims Asthma is a heterogeneous disease with poorly defined sub-phenotypes lacking clear aetiology. Our aim was to investigate the utility of urinary metabolomics for identifying asthma sub-phenotypes. Methods Urine was collected from 613 participants across 15 international sites for the European U-BIOPRED project (www.ubiopred.eu). The cohort comprised healthy controls (HC, n=108), mild-to-moderate asthmatics (MMA, n=87), and severe asthmatics, including non-smokers (SAns, n=310) and smokers (SAs, n=108). Longitudinal sampling was performed for SAns (n=225) and SAs (n=80). Untargeted metabolomic profiles were acquired in 17 analytical batches using LC-QTOF-MS, with HILIC chromatography, using simultaneous full scan MS and DIA at 2 collision energies. Pooled QC samples were injected after every 5th sample. Metabolites were identified using an in-house MS/MS library of 413 metabolites. Batch-drift was corrected using the QC-RSC algorithm. Data were analysed using Principal Components–Canonical Variate Analysis (PC-CVA), Topological Data Analysis (TDA), and Hierarchical Clustering Analysis (HCA). Results 90 metabolites were reproducibly detected (average QC-RSD=3.3%); 43 changed significantly in asthma (FDR<0.05). PC-CVA showed that HC and MMA differed significantly from SA (p=1.4x10-14), and SAns differed significantly from SAs (p=0.003). HCA and TDA revealed sub-phenotypes of SA associated with tryptophan metabolism dysregulation; blood cell transcriptomics corroborated these findings. Conclusions Urinary metabolomics successfully identified metabolic profiles associated with asthma severity. While observed fold-changes were low, study power, experimental design, and strict QC measures enabled the detection of statistically robust metabolic alterations. Tryptophan metabolites are implicated in T-cell differentiation, cytokine activation, and oxidative stress, which may represent a useful strategy for sub-phenotyping asthma.

O-184	Malaria parasite metabolism is supported by host derived nutrients obtained through RhopH2 mediated New Permeability Pathways

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Malaria is a major global health problem, with over 200 million reported cases and an estimated 429,000 deaths in 2015. Plasmodium parasites, which invade erythrocytes and cause malaria, drastically modify their host cells to render them permeable for exchange of nutrients and waste with the blood plasma via the formation of new permeability pathways (NPPs). Defining the molecular makeup of the NPPs is critical for identifying the best application strategies for new anti-malarial drugs and understanding the mechanisms by which malaria parasites can alter NPPs to develop resistance to chemotherapeutic agents. In this study, using untargeted comparative metabolomics, we investigated the role of a parasite encoded protein, RhopH2 in Plasmodium-infected erythrocytes. We found that knockdown of RhopH2 expression leads to a depletion of essential vitamins and cofactors such as folate and thiamin phosphates and decreased de novo synthesis of pyrimidines. This causes a significant defect in parasite growth, replication and subsequent transition into the next cell cycle. A similar metabolic and phenotypic effect was observed when wild type parasites were treated with a chemical inhibitor of the NPPs. For further validation, using uptake assays with different substrates, we confirmed that the import of metabolites known to enter Plasmodium infected erythrocytes via the NPPs is also reduced in RhopH2 knockdown parasites. RhopH2 was also found to interact with other proteins involved in host cell remodeling. These findings provide direct evidence for the contribution of RhopH2 in the formation of NPPs and highlight their importance in parasite metabolism.

O-197	BIOMARKER DISCOVERY FOR HEPATITIS C PROGRESSION USING METABOLOMICS

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OBJECTIVES: Little is known about how Hepatitis C (HepC) infection progresses to Hepatocellular Carcinoma (HCC) in patients with advanced fibrosis. The objective of this study is to use Liquid Chromatography (LC) based Mass Spectrometry (MS) techniques to determine metabolites that are differentially expressed between Stage IV HepC infected individuals and individuals co-infected with both Stage IV HepC and HCC. We also aim to use the results from the metabolomics experiments to create a biomarker panel, using machine learning algorithms, for the early diagnosis of HCC. METHODS/STUDY POPULATION: LC/MS-MS metabolomics experiments were performed on serum samples from 30 Stage IV HepC and 30 Stage IV HepC, HCC co-infected individuals. Using statistical hypothesis tests, significantly different metabolites between the two groups at the 0.05 level of significance were identified. Using those results, machine learning algorithms were employed to create a biomarker panel which can be used to predict early stage HCC infection. RESULTS: Several metabolites are significantly different between the two groups. Machine learning algorithms were used to identify a panel of potential biomarkers which can be used in the future to identify Stage IV HepC individuals which have progressed to early-stage HCC. DISCUSSION: HCC is the third most common cause of cancer deaths worldwide, yet there is no reliable diagnostic test for the early detection of the disease, when treatment options that improve long-term survival and patient outcomes are available. The discovery of a biomarker panel for the detection of early-stage HCC should provide new metabolite targets for diagnostic assays.

O-203	Metabolic Reprogramming Induced by Retinoic Acid Ameliorates Desmoplasia in Pancreatic Cancer

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Epithelial to mesenchymal transition (EMT) has been known to be integral to cancer metastasis. TGF? is a key signaling mediator that is involved in EMT and plays a pivotal role in increased production of extra cellular matrix (ECM) proteins that result in desmoplasia in PDAC. An in vitro metabolomics profiling approach was used primarily to confirm the identity of the core signaling pathways altered by TGF?. The unbiased view of metabolite abundance was studied to reveal the existence of unsuspected metabolic changes. A striking observation from metabolic phenotyping was the dysregulation of the signaling molecule, retinoic acid (RA) that was found to be significantly elevated in PANC1 cells treated with TGF? compared to untreated cells. We hypothesized that targeting retinoic acid receptor signaling, would cause rearrangement of the ECM components through metabolic reprogramming. Hence we investigated if retinoic acid signaling could be modulated to alleviate fibrosis in in vitro PDAC model through remodeling of the ECM components. The results we obtained using orthogonal methodologies shows that inhibition of retinoic acid signaling by specific retinoic acid receptor antagonist causes alterations in hexoseamine biosynthesis (HSB) pathway as well as extracellular matrix (ECM) remodeling. Targeted analysis of metabolites participating in the HSB pathway and their role in EMT mediated desmoplasia in PDAC is ongoing in addition to untargeted metabolomic profiling cell lines treated with retinoic acid and its receptor antagonists. Our methodology underscores the power of high through put metabolomics technology for novel discoveries that further our understanding of cancer progression and metastasis.

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O-222	Contaminants removal from tissue samples using DESI Ion Mobility imaging

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Desorption electrospray ionization (DESI) imaging is an ambient ionization technique that requires little to no sample preparation prior to analysis of surface analytes. DESI imaging has been demonstrated to accurately distinguish between cancer and normal tissue types, based on the differences in metabolic profiles. However, these studies are limited to fresh frozen, polymer-free samples, which are not always readily available. An alternative to these are fresh frozen samples embedded in optimal cutting temperature medium (OCT), which are widely available in most pathological laboratories. OCT is a common embedding medium, which consists of water-soluble glycols and resins and is used for sectioning fresh frozen specimens. OCT-embedded samples cause issues in positive ion mode DESI imaging, due to overlap in mass peaks between the metabolites from samples and polymers from OCT. Ion mobility mass spectrometry adds an additional dimension of separation based on the collisional cross-section (CCS) of ions. It is therefore able to separate isobaric peaks with different structures. As a proof of concept, both pork liver embedded in OCT and pork liver embedded in water were imaged with DESI, using a high resolution mass spectrometer equipped with ion mobility. The acquired images have demonstrated that the addition of ion mobility was able to separate overlapping OCT and lipid signals (e.g. 789.43 m/z and 789.46 m/z) based on their CCS values. This allowed a complete removal of interfering polymer peaks and provided comparable spectra for both embedding types.

O-230	Metabolomics Approach in Tohoku Medical Megabank Project Prospective Cohort Studies

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Tohoku Medical Megabank Organization has been conducting the Tohoku Medical Megabank (TMM) Project, which operates two prospective cohort studies in Japan: the TMM Community-Based Cohort Study and the TMM Birth and Three-Generation Cohort Study. We have already recruited more than 150,000 participants and will start the follow-up health assessments from April 2017. Using the biospecimens and information collected from their participants, we have been performing genome and omics research. We have already determined the concentration distributions of metabolites in more than 1,000 Japanese plasma samples, using both mass spectrometry (MS) and NMR spectroscopy techniques. These results have been released as a database, Japanese Multi Omics Reference Panel (jMorp), which is freely available in our Web site (https://jmorp.megabank.tohoku.ac.jp/). This database is the first open database of Japanese standard omics information and is used as the reference data for a wide variety of medical and pharmacological studies. We also investigated the correlation of these omics data with blood test values, items of questionnaires, and genome variants and found many kinds of correlations among them. In order to expand our omics database, we are operating metabolome analysis for more than 5,000 plasma samples and will release high precision jMorp database and the results of the correlation analyses near future.

O-237	Molecular mechanisms of lung injury during cardiac surgery

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Introduction: Acute lung injury is among the leading causes of morbidity and mortality in patients undergoing cardiac surgery with cardiopulmonary bypass (CPB). Exposure of blood to the CPB circuit and lung ischemia-reperfusion leads to lung injury with increased microvascular permeability, pulmonary oedema, and inflammation. Currently, the pathophysiological mechanisms are not completely understood. Aim: The aims of this study are to identify intraoperative metabolic alterations, to relate them to the alveolar cellular immune system, and to elucidate the possible mechanisms of postoperative lung injury. Methods: To evaluate cellular immune system and metabolic events in the lungs, bronchoalveolar lavage fluid (BALF) was collected from 49 surgical patients before and after CPB. BALF was screened for macrophages, neutrophils, lymphocytes, eosinophils (CellaVision DM96), and metabolites (1H Nuclear Magnetic Resonance spectroscopy). Multivariate unsupervised and paired analyses were applied to identify differences between samples, while Spearman correlation test was used to detect molecular associations. Statistical significance was defined as a p-value ?0.05. Results: Profound changes were identified in cells and metabolites at the end of CPB, indicating increased molecular activity in the alveoli during surgical procedure. Fold change analysis revealed changes in energy metabolites (>3-fold increased, p<0.00001), amino acids (>2-fold increased, p<0.01), phospholipids (>3-fold increased, p<0.001), and polyamines (>24-fold increased, p<0.00001). Metabolites were linked to activated metabolism in BALF cells, but also to vascular permeability, pulmonary oedema, proteolytic activity, and oxidative stress. Conclusion: We provide a comprehensive pulmonary profile of immunologic and metabolic events occurring during CPB and identify possible pathophysiological mechanisms to lung injury.

O-239	A robust analytical pipeline for high throughput molecular phenotyping of populations

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The global growth of molecular epidemiology has necessitated a step change in molecular profiling technologies for delivering high throughput spectral data defining population phenotypes. The MRC-NIHR National Phenome Centre (NPC) is one of the first centres dedicated to large-scale metabolic profiling and was opened in Imperial College. A key objective of the NPC is to establish and validate analytical methods for large sample cohorts. We have developed and optimised high quality and robust NMR-based profiling approaches for large-scale metabolic phenotyping and applied it to approximately 8000 urine samples from 7 independent studies analysed across 4 years to assess potential confounding variation associated with experimental quality, sample stability and biological variation (e.g. gender and age). Within each study, two different quality control (QC) samples (one internal to the study and an inter-study comparator) were included allowing both intra and inter-study variability to be defined. The coefficient of variation of the urine metabolic profile of the QC samples within a single study was below 0.1 for more than 90% of the metabolites. However inter-study analysis showed that while some metabolites such as creatinine or hippuric acid or even histidine present a very low coefficient of variance (CV<0.1) others like citrate, dimethylamine, or acetate present higher variation (CV~0.5). Using QC samples to define confidence limits for spectral stability we applied our optimised analytical pipeline to characterise variation attributed to gender and age and to define "normal" reference ranges for representative metabolites involved in endogenous metabolic pathways and gut microbial metabolism.

O-241	The effects of changing the liquid/solid content of an isoenergetic test meal on gastric emptying and the blood lipidome

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CO-AUTHORS: *Evelina Charidemou, Elise Orford, Sara Wassell, Mathew Harvey, Michelle Venables, Julian Griffin*

Gastric emptying (GE) is a term describing the process by which food leaves the stomach. This study investigates whether the liquid, carbohydrate rich, component of a mixed meal delays GE of the solid portion and induces changes in the plasma lipidome. Four of eight healthy male participants have been recruited to date in this randomised, 5-way crossover study. On each study visit, fasted participants received one of the five isoenergetic mixed meals (2MJ) labelled with [1-13C] octanoic acid. The meals included: Standard (with or without bread), high fat, high carbohydrate and high protein. Breath and plasma samples were collected at set intervals for 6 hours. Breath samples were analysed by Isotope Ratio Mass Spectrometry (IRMS). Plasma samples were examined by LC-MS based lipidomics. The GE parameters including half emptying time (Thalf, the time taken for 50% of the total 13C label excreted to appear in the breath) and lag time (Tlag, the time of maximum excretion rate of label in the breath) were analysed using WinBugs. There was no difference found in isotope recovery or Thalf between the meals, while Tlag was meal dependent (c2, Friedman=10.4, p=0.034). However, there was no significant difference in Tlag, Thalf(in) and Tlag(in) between meals under pairwise comparison with post-hoc Bonferroni correction. The lipid data were analysed using OPLS-DA. Three different groups were classified with the high carbohydrate meal separated most from the others (R2=0.61, Q2=0.74). This ongoing study demonstrates the use of stable isotope technology to follow physiological changes while profiling changes in lipidome.

O-244	Rapid characterisation of the vaginal metabolome during pregnancy using desorption electrospray ionization (DESI) MS medical swab analysis

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Sequencing based characterisation of vaginal bacterial communities (VBCs) have limited clinical application due to time and cost constraints and the failure to capture host-microbiota interactions. We recently developed a novel approach for rapid characterisation of mucosal metabolic profiles directly from medical swabs (Pruski et al. 2017) using desorption electrospray ionization mass spectrometry (DESI-MS). This method permits rapid assessment of VBC-related metabolic changes associated with bacterial vaginosis, a common condition characterised by reduced levels of commensal Lactobacillus species and overgrowth of anaerobic bacteria. In this study, we use DESI-MS medical swab analysis to characterise the vaginal metabolome in a cohort of 63 women longitudinally sampled (n=248 swabs) throughout healthy pregnancy. Relationships between maternal factors (gestational age, BMI, vaginal pH) and metabolite changes occurring across gestational age were assessed. DESI-MS profiles were compared to reversed phase LC-MS profiles and were then correlated with paired vaginal microbiota profiles (by Bacterial 16S rRNA gene sequencing) to identify discriminative features predictive of VBC composition. The significant features (including SCFA, polyamines and lipids) were compared with metabolite profiles obtained from purified cultures. A Random Forest classifier with cross validation was applied to assess the prediction accuracy of a healthy vaginal community (Lactobacillus dominated) and a dysbiotic state (Lactobacillus depleted) achieving overall > 80% accuracy using DESI MS. These results indicate that DESI MS analysis of medical swabs fulfills the criteria for a routine diagnostic procedure and may permit identification of vaginal dysbiosis in pregnancy and early stratification of women at risk of infection-related pathologies during pregnancy).

O-245	Aiding diagnosis of rare disease: applications of mass spectrometry-based metabolomics in the Undiagnosed Diseases Network

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In the U.S., 6% of the general population suffers from a rare disorder that has evaded diagnosis. The goals of the NIH Undiagnosed Diseases Network (UDN) include improving the level of diagnosis and care for patients and facilitating research into the etiology of undiagnosed diseases. As the UDN Metabolomics Core, we are performing MS-based metabolomics and lipidomics analyses of plasma, cerebrospinal fluid (CSF) and urine from patients and first degree relatives, as well as disease models in organisms such as Drosophila and zebrafish. These data are being compared against similar metabolic profiles from healthy individuals and in integrative analyses together with results from patient gene sequencing. To allow for proper statistical analyses of UDN patient data, we generated reference datasets from analyses of plasma, CSF, and urine from >391 individuals with no known metabolic disease and representative of the demographics of the UDN, all driven by power analyses of historical data. To date, we have performed >1500 untargeted metabolomics and lipidomics analyses of plasma, CSF, and urine samples, resulting in reference datasets containing >300 identified and unidentified metabolites and >500 identified lipids as a community resource. A total of 145 samples (83 plasma, 57 urine, and 5 CSF) from 83 UDN patients and first degree relatives have been analyzed, and the resulting data compared to the appropriate reference datasets in order to identify outlier metabolites and lipids. This data is being used to identify the metabolic pathways that have been affected by the underlying disease processes.

O-279	DMGV is a Novel Marker of Liver Fat and Predicts Future Development of Type 2 Diabetes

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Background: Unbiased "non-targeted" metabolite profiling techniques hold considerable promise for biomarker and pathway discovery, though there have been few successful applications to human disease. We aimed to uncover novel biomarkers of non-alcoholic fatty liver disease (NAFLD) and cardiometabolic disease using non-targeted metabolomic profiling in well-phenotyped human cohorts. Methods: Non-targeted metabolite profiling was performed using hydrophilic interaction chromatography on a UHPLC Q-Exactive hybrid orbitrap LC/MS system. Results: The non-targeted platform was applied first to plasma of a discovery cohort of 1066 participants, of whom 470 had CT-defined fatty liver disease. An unknown metabolite with a mass/charge ratio of 202.1185 had the strongest association with CT-defined liver fat, which was quantified using the liver-to-phantom ratio (P=1.16E-10, adjusting for age, sex, smoking, alcohol consumption, HDL and triglyceride concentration, HOMA-IR, hypertension, and BMI). Following structural elucidation by LC-MS and the synthesis of a chemical standard, we confirmed the peak identity as ?-keto-?-(NGNG-dimethylguanidino)-valeric acid (DMGV). DMGV was significantly associated with non-alcoholic steatohepatitis (NASH), in a cohort of 36 patients with biopsy-proven NASH and 36 age, sex, and BMI-matched controls (OR=1.96, P=0.011, conditionally adjusted for age, sex, and BMI). Further, DMGV was predictive of future type 2 diabetes mellitus (T2D) in 196 cases of incident diabetes and 126 controls in the Malmo Diet and Cancer Study (OR=1.56, P=8.6E-4, adjusting for age, sex, glucose, and BMI; mean follow-up period of 12.8 years). Conclusions: DMGV is a novel marker of NAFLD and NASH, and is a predictor of future T2D.

O-281	Metabolomic analysis of the effects of a ten week high protein diet in older men

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Epidemiological evidence suggests that a higher protein diet may be beneficial for optimal health, particularly for the elderly. However, the impact of the current protein recommended dietary allowance (RDA; 0.8 g/kg body weight per day) versus a higher protein intake on metabolomic profiles in the elderly has not been established. To investigate this, we prepared isocaloric omnivorous diets containing either the RDA or twice the RDA (2RDA) of protein. These were allocated to two groups of 15 healthy free living men (74 ± 4 years) and fed for 10 weeks. Blood samples were collected in the overnight fasted state prior to and immediately following the 10 week intervention. Non-targeted analysis of polar metabolites in blood plasma was performed by LC-MS (HILIC chromatography coupled to an Exactive™ mass spectrometer). Despite that the diets were designed to balance energy needs, body mass decreased in the men consuming the RDA, but was maintained in the 2RDA group (-2.0 ± 2.4 kg, p<0.01 vs. -0.1 ± 2.2 kg, p>0.05). Metabolite profiles were altered by dietary intervention in both groups. There were increases in glutamine (p<0.001) and asparagine (p<0.05), and reductions in glutamic acid (p<0.001) and hydroxyproline (p<0.005). Alanine and glycine plasma concentrations increased after the RDA diet but decreased after the 2RDA diet (group × time interaction p<0.05 each). Ten weeks of controlled dietary intervention elicited marked alterations in circulating metabolite profiles, with analysis on-going to identify the major biochemical pathways influenced by an increased protein intake.

O-289	Plasma metabolites associated with type 2 diabetes in a Swedish population - a nested case-control study

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The discovery of early metabolic alterations of type 2 diabetes (T2D) may provide novel insight into the pathophysiology of T2D and improve disease prediction. We established a nested case-control study within the Västerbotten Intervention Programme cohort to discover plasma metabolites associated with risk of developing T2D. Using untargeted LCMS metabolomics, we analyzed plasma samples from 503 case-control pairs at baseline (median time of 7 years prior to T2D diagnosis) and investigated change of metabolites over time in 10-year follow up samples from 187 case-control pairs. A total of 46 discriminative metabolites between cases and controls at baseline were unbiasedly selected using a comprehensive data-analysis pipeline adapted for large-scale metabolomics. After adjusting for BMI, fasting glucose and lifestyle factors, diglycerides, a bile acid, branched-chain amino acids (BCAA) and their catabolic metabolites were associated with increased risk of T2D, whereas phosphocholines (PCs), N-Acetylglycine, and 2-hydroxyethanesulfonate were associated with decreased risk. These metabolites correlated strongly with insulin resistance and/or beta cell dysfunction, suggesting specific roles in disease pathophysiology. Moreover, PCs containing odd-chain fatty acids, BCAAs and the bile acid had high long-term reproducibility among healthy controls, and their changes over time reflected disease progression in cases. Furthermore, optimal utilization of predictive metabolites and traditional risk factors significantly improved T2D risk prediction, as assessed by area under the receiver operating characteristic curve, discrimination improvement index, and net reclassification index. The results underscored the ability of single measurements of predictive metabolites to reveal pathophysiology and to improve risk prediction several years before onset of T2D.

O-300	LIPIDOMICS REVEALS CEREBROSPINAL-FLUID SIGNATURES OF ALS

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Objective The objectives of this study were to investigate the cerebrospinal fluid (CSF) lipidomic signature of ALS patients to evaluate the diagnostic and predictive values of the profile and to identify pathophysiologic biomarkers. Methods We performed an untargeted lipidomic analysis in 40 ALS patients compared to 45 controls. We robustly determined 122 lipids by liquid chromatography coupled to high-resolution mass spectrometry. Parameters of disease progression were collected at baseline and again one year later (ALSFRS-r, FVC, BMI°, as well as survival. Lipidome profiles were then subjected to powerful statistical modelling to compare ALS and controls and to model the rate of progression. Results ALS displayed a highly significant specific CSF lipidomic signature involving phosphatidylcholines, sphingomyelins and triglycerides. Phosphatidylcholine PC(36:4), higher in ALS (p=0.0003) was the strongest biomarker. Analysis of lipids in the brain cortex of ALS mice confirmed the role of some discriminant lipids such as PC. We also obtained an excellent model (accuracy of 79%) for predicting the variation of the ALSFRS-r score from baseline lipidome. Significant predictions of clinical evolution were found to be correlated to sphingomyelins and triglycerides with long-chain fatty acids. Interpretation Our study, which shows extensive lipid remodelling in the CSF of ALS patients, provides new biomarkers of the disease and its evolution. Importantly, the lipidomic signature found in ALS patients consistent with ALS mice findings, highlighted phosphatidylcholines, that merit to be further explored.

O-314	Enrichment of Secondary Bile Acids associated with Food Allergy in Healthy Infant Cohort

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Rationale: Food allergy is a life threatening condition, and while metabolomic analyses have been used in murine models of food allergy, metabolomics studies of food allergy in humans remain limited. Methods: We examined plasma metabolomics from a cross-sectional study of infants recruited for the VDAART (N=213) prenatal vitamin D trials. We compared the 32 children with self-reported food allergy to those without (n=181). We employed logistic regression to identify the top metabolites associated with food allergy in the infant cohort, and replicated these in a cohort of Costa Rican asthmatic children with self-reported food allergy (N=243, 38 with self-reported food allergy). We also conducted receiver operator curve (ROC) analysis (adjusting for gender, race, and age) to determine the utility of our top hits in predicting food allergy. Results: We identified 24 metabolites (p<0.05) associated with food allergy. Secondary bile acids represented 4 of the top 8 metabolites, including ursodeoxycholate (OR:4.51, 95%CI:2.0-11.7, p=0.0009), glycoursodeoxycholate (OR:2.70, 95%CI:1.29-6.11, p=0.012), isoursodeoxycholate (OR:2.32, 95%CI:1.19-4.80, p=0.018), and glycodeoxycholate (OR:1.70, 95%CI:1.10-2.7, p=0.019). We also found an association with deoxycholate and food allergy in our Costa Rican cohort (p=0.0095). ROC analysis using the top 24 metabolites yielded an area under the curve (AUC) of 0.86 (p=1.9x10-8), and using the 4 deoxycholates yielded an AUC of 0.76 (p=0.00098). Conclusions: These results suggest that there exist metabolite profiles of pediatric food allergy that may prove useful as biomarkers. In particular, the strong association of secondary bile acids suggests a possible role for these metabolites in the pathophysiology of food allergy.

O-319	An integrated approach combining metabolomics, lipidomics and glycomics on plasma to highlight metabolism dysregulation in hepatic encephalopathy

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Hepatic encephalopathy (HE) is a neurological complication observed in patients with liver disease, mainly cirrhosis. About 50% of cirrhotic patients will develop HE with symptoms ranging from mild cognitive impairment to coma. Pathophysiological mechanisms remain poorly understood despite a possible role of hyperammonemia. This limits the discovery of new preventive and therapeutic strategies. In this context, untargeted metabolomics can be useful to understand the dysfunction of metabolic pathways in HE patients. A previous study performed on cerebrospinal fluid highlights the dysregulation of energy metabolism1. To complete this work, we focus on plasma and perform a "multi-omics" study including metabolomics, lipidomics and glycomics. Metabolomics and lipidomics experiments were performed using liquid chromatography coupled to high resolution mass spectrometry. N-glycan analysis was performed using a MALDI-ToF/ToF instrument. Plasma samples were collected from 12 cirrhotic patients with HE, 13 cirrhotic patients without HE and 9 healthy controls. In addition to the 122 metabolites found in CSF, 190 other metabolites were identified in plasma. In lipidomics, more than 900 lipids were annotated while around 50 N-glycans were detected. Modifications in metabolite concentrations between the cirrhotic and control groups were observed. Moreover, this study highlights the specific modifications in HE samples compared to cirrhotic ones. In lipidomics, the main impacted families are glycerophosphocholines, sphingomyelins and cholesteryl esters with lower concentrations in HE plasma. Metabolomics supports these observations (e.g. diminution of sphingosine-1-phosphate). Glycomics results are currently being analyzed. The combination of the different techniques will provide new complementary insights into HE metabolism dysregulation. 1: J Hepatol.,2016,65:1120

O-324	Metabolic reprogramming of macrophages exposed to Pseudomonas aeruginosa biofilm

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Currently, the annual economic cost of chronic wounds exceeds $1 billion in the United States, and the incidence of non-healing human wounds is expected to dramatically increase in the next several years due to emerging Type 2 diabetes epidemics. Several common characteristics typical of chronic wounds include tissue colonization by persistent antibiotic-resistant pathogenic microbial biofilms, excessive inflammation, and failure of human cells to resolve the wound. Pseudomonas aeruginosa is one of the predominant opportunistic pathogens that colonizes greater than 50% of all chronic wounds in the U.S, and is a serious health threat.To better understand the molecular processes by which P. aeruginosa biofilms interfere with human macrophage immune responses, we have undertaken nuclear magnetic resonance-based metabolomics studies of activated and resting macrophages. The studies aim to probe the metabolic reprogramming of these immune cells as result of exposure to secreted molecules produced by P. aeruginosa biofilms, using an in vitro host-pathogen co-culture model. Herein, we present our most recent NMR-based metabolomics results demonstrating the presence of a significant metabolic shift between different macrophage phenotypes. This metabolic phenotyping is correlated with fluorescence-activated cell sorting (FACS) analysis which has been employed to characterize the relationship between metabolic profiles and macrophage immunomodulation (i.e. macrophage polarization into pro- or anti-inflammatory M1 or M2 subpopulations, respectively), resulting from macrophage exposure to secreted molecules from co-culture P. aeruginosa biofilms. The overall research goals are to generate foundational knowledge for the design of new, metabolism-based, therapeutic interventions to treat biofilm-infected human chronic wounds.

O-341	The metabolomic analysis of sequential hair segments reflected the metabolic changes of normal, small-for-gestational-age, and gestational diabetes mellitus affected pregnancies across the trimesters.

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Background: Metabolomics is an increasingly popular analytical approach for the prediction, diagnosis, and prognosis of pregnancy outcomes. Biological specimens such as blood and urine provide a transient biochemical profile. In contrast, hair is a stable specimen which can provide a long-term metabolite profile containing information from both endogenous compounds and exogenous compounds. We hypothesised that the metabolomic analysis of hair segments could be used to study the longitudinal metabolite profile of normal pregnancies and pregnancies complicated by gestational diabetes mellitus (GDM) or resulting in a small-for-gestational-age infant (SGA). Methods: Hair samples were collected from 170 women in Auckland, New Zealand at 34-36 weeks' gestation. The hair was segmented based on trimester and each segment was analysed using both gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. Repeated measures ANVOA, logistic regression (adjusting for BMI, maternal age and ethnicity), and a Pathway Activity Profiling R package were applied to determine which hair metabolites/ metabolic pathways differed significantly across trimesters as well as between normal and adverse pregnancy outcomes. Results: The hair metabolome and metabolic pathways identified were significantly different between trimesters. The majority of metabolites associated with SGA were saturated fatty acids such as myristate and margarate, observed in hair from the second trimester. Metabolites associated with GDM were involved in the central carbon metabolism, and were observed in hair from the third trimester. Conclusion: Our results demonstrated that hair segments could be used to reflect the longitudinal changes of the metabolite profile as well as to differentiate pregnancy complications from healthy pregnancies.

O-342	Pre-pregnancy maternal obesity can result in metabolic dysregulation and memory impairment in male offspring

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Background: Obesity is a worldwide epidemic which is associated with ranges of health problems. There is increasing evidence that offspring health during childhood and later adult life is affected by maternal obesity. We hypothesized that the offspring of mice from an obese mother, when following a high-fat diet (HFD) after weaning, would exhibit metabolic dysregulation and memory impairment. Methods: In our pilot study a LepRdb/+ mouse model (n=37) was used to model pre-pregnancy maternal obesity, and the c57bl/6 wildtype (n=36) was used as a control group. Offspring were fed either a HFD or a low-fat control diet (LFD) after weaning (between 8-10 weeks). The Morris water maze was performed between 28-30 weeks. Metabolomic profiles of serum, liver, ovary, testis, kidney, and brown adipose tissue from each group collected at the age of 30-32 weeks, were analyzed using Gas Chromatography-Mass Spectrometry. Results: The offspring metabolomic profiles from the serum and organs (with exception of the kidney) were significantly different between the HFD-group and LFD-group. Maternal obesity only influenced the metabolomic profiles of serum, ovary, testis, and male brown adipose tissue in their offspring, when compared to the control group. The memory of male offspring from obese maternal mice was significantly impaired, while no significant differences were observed in female offspring. Conclusion: Our findings suggest that offspring from obese maternal mice that consume a HFD after weaning have significantly altered metabolomic profiles, and memory was significantly impaired in male offspring. Further research is needed to validate the results of our pilot study.

O-350	Insufficient Sleep Alters Plasma Metabolites Linked to Insulin Resistance and Diabetes Risk

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CO-AUTHORS: *Rachel Markwald, Charmion Cruickshank-Quinn, Kevin Quinn, Nichole Reisdorph, Kenneth Wright*

Introduction: Many people in modern society sleep less than the recommended 7h/night and insufficient sleep is associated with increased diabetes risk. We previously reported a 5-day simulated work-week of insufficient sleep decreased insulin sensitivity. To identify potential biochemical mechanisms underlying such metabolic dysregulation, we investigated the plasma metabolome before and during insufficient sleep. Methods: We conducted a randomized cross-over clinical laboratory study where 16 healthy subjects aged 22.4±4.8y (mean±SD) completed 3 baseline days (9h sleep opportunity/night) followed by 5 day insufficient (5h/night), and adequate (9h/night) sleep conditions. At baseline, food intake was designed to meet energy requirements, whereas during insufficient and adequate sleep, food intake was ad-libitum. Subjects completed oral glucose tolerance tests at baseline and during adequate and insufficient sleep conditions for assessment of insulin sensitivity. We analyzed plasma by untargeted LC/MS every 4h across the final 24h of baseline, insufficient, and adequate sleep. Results: ANOVA (false discovery rate < 0.2) and random forest analyses were used to identify the top metabolites differentiating insufficient and adequate sleep. In total, 241 metabolites (~5%) changed between conditions including glycerophospholipids, sphingolipids, diacylglycerols, triacylglycerols, and acylcarnitines. Specifically, acylcarnitines C10:1 and C10:2 were decreased, and short-chain triglyceride species (TAG-42:0, TAG-46:1, TAG-48:2) were elevated during insufficient sleep. Conclusion: We found insufficient sleep broadly affects the human plasma metabolome. Changes in plasma acylcarnitines and elevated short-chain triglycerides during insufficient sleep are consistent with altered fatty acid oxidation. Thus, our findings suggest insufficient sleep alters fatty acid oxidation and contributes to reduced insulin sensitivity, elevating diabetes risk.

O-356	How Blood Removal Affects Exercise Performance and the Plasma Metabolome

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Purpose: Blood removal has both haematological and non-haematological affects resulting in alterations to aerobic and anaerobic metabolism and overall exercise performance. This study examined the longitudinal effects (42 days) of whole blood removal on aerobic performance, and the plasma metabolome, in a "trained" and "non-trained" cohort. Methods: At baseline and 7 additional time points up to and including 42 days, 470 mL of whole blood were removed from 8 trained male cyclists, and 7 healthy untrained males. At all time points, plasma samples were collected and stored at -80°C and aerobic performance was assessed using an incremental cycling test (VO2max) and 4-minute self-paced cycling time trials. Plasma samples were analysed using LC-TOFMS (ESI +/-) employing C18 chromatography. Pooled QC samples were injected after every 5th sample. Data were processed using XCMS and analytical drift was corrected using the QC-RSC algorithm. Data were analysed using Repeated Measures ANOVA, Principal Components – Canonical Variate Analysis (PC-CVA) and Hierarchical Clustering Analysis (HCA). Results: VO2max decreased by 6±12%, 2±6%, 1±2% at 24 h, 7 d, and 21 d, respectively. 147 peaks changed significantly (p<0.001) with time, but no differences were observed between study groups. PC-CVA and HCA corroborated these findings. HCA clustered metabolites into 8 different trajectories. Conclusion: Many dysregulated metabolites mapped closely to the expected return to homeostasis; however, a number of metabolites appear to remain perturbed beyond the return of aerobic performance. These findings provide insight into the biological understanding for the effects of blood manipulation in trained and healthy individuals.

O-366	Exploring dormant genital tuberculosis associated infertility - 1H NMR based serum lipidomics approach

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CO-AUTHORS: *Elavarasan Subramani, Mamata Joshi, Chaitali Datta Ray, Baidyanath Chakravarty*

Genital tuberculosis (GTB) is an important clinical problem causing infertility in women and is an underdiagnosed type of extra-pulmonary tuberculosis. As an intracellular pathogen, Mycobacterium tuberculosis strongly influences the metabolism of host cells, potentially inducing dysregulation in lipid metabolites. This motivated us to explore the significantly altered lipid molecules that may enhance our understanding of the underlying host-pathogen interaction.Unexplained infertile women having at least 3 IVF failure with (n=26) and without dormant GTB (n=26) were recruited for this study. Serum samples were collected on day 7–10 post-ovulation from all subjects at the Institute of Reproductive Medicine, Kolkata, India. 700 MHz proton NMR spectra of serum lipid fractions were recorded for both the groups. Multivariate and univariate analyses were applied to all spectra. Lipids including C26H3 and C27H3 free/esterified cholesterol, C19H3 free and C19H3 esterified cholesterol and CH2CO 22:6 fatty acids were found to significantly down-regulated in serum of women with dormant GTB compared with controls. Further, significant upregulation of serum free fatty acid residues such as CH2CO FA, CHCH2(CH2)n FA and(CH2)n FA and triglycerides in dormant GTB patients was found as compared to controls. This study reports, for the first time, a clear lipidomic differentiation between women with dormant GTB and controls during window of implantation. The dysregulated lipids observed in the present study are indicative of poor endometrial receptivity in these women. These findings may be explored further by clinicians for better therapeutic management of patients with GTB related infertility.

O-381	Integrated omics profiling of NGLY1 deficiency disease

PRESENTING AUTHOR: *Songjie Chen, Stanford University, United States*

CO-AUTHORS: *Guangwen Wang, Christine Yeh, Sharon Pitteri, David Marciano, Michael Snyder*

Patients of rare diseases not only suffer severe pathology, but also insufficiently diagnosed and cured due to the insufficient pathological information and variations of symptoms. Metabolomic profiling of patients' biofluids provides broad information of the affected metabolic pathways, however, individual metabolome exhibits extensive variation due to genetic background and environmental factors. We generated induced pluripotent stem cells (iPSCs) from patients of a rare disease, NGLY1 deficiency, and corrected pathogenic mutations by CRISPR to provide superior controls for the characterization of disease relevant molecular phenotype. We further applied metabolomic profiling integrated with proteomics analysis of iPSCs and patients' plasma to capture the disease related molecular phenotype. The integrated personalized omics profiling of cell models generated from NGLY1 deficiency patient are expected to discover the metabolites and proteins involved in NGLY1 deficiency affected pathways. An on-dish platform generated from the cell models and captured molecular phenotypes will be further applied for the screening of potential therapies. Such an integrated personalized approach developed in this project will have translational impact on the research of diverse rare diseases.

O-384	Multi-omics analysis reveals the biochemical changes associated with artemisinin-resistant malaria parasites

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CO-AUTHORS: *Ghizal Siddiqui, Adrian Russell, Anubhav Srivastava*

Malaria is a major global health concern, responsible for over 200 million cases and 429 000 deaths annually. Artemisinin combination therapies are the first-line treatment for malaria, and few effective alternatives exist. Alarmingly, the emergence and spread of artemisinin resistance in South-East Asia threatens our ability to treat malaria, and the mechanism of this resistance is poorly understood. Artemisinin resistance has been associated with mutations in the PfKelch13 gene of the malaria parasite. However, the function of PfKelch13, and the mechanism of resistance remain unknown. The aim of this study was to reveal the biochemical impact of common PfKelch13 mutations using metabolomics, peptidomics and proteomics analysis. Two field-derived parasite strains bearing PfKelch13 mutations associated with artemisinin resistance (Cam3.IIR539T and Cam3.IIC580Y), and an isogenic PfKelch13 wild-type control (Cam3.IIRev), were cultured in vitro and metabolites, peptides and proteins were extracted in parallel for analysis. Proteomics analysis with dimethyl labelling and nanoLC-MS revealed the abundance of PfKelch13 itself to be lower in the mutant lines. Metabolomics analysis using a HILIC-Orbitrap LC-MS platform revealed few significant overall differences in metabolite levels, with significant accumulation of glutathione and gamma-glutamylcysteine observed in the PfKelch13-mutant strains. Peptidomics analysis, using reversed-phase nanoLC-MS, demonstrated a depletion of haemoglobin- derived peptides, suggesting decreased haemoglobin digestion by the mutant parasites. Artemisinins are thought to be activated by haemoglobin-derived iron to produce free radicals that are responsible for their antiparasitic activity. This study suggests that PfKelch13-mutant parasites may overcome this effect by a combination of reduced haemoglobin digestion and enhanced glutathione production.

O-436	Comprehensive High-Throughput Targeted Lipidomics

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CO-AUTHORS: *Christopher Barlow, Natalie Mellett, Jacquelyn Weir, Peter Meikle*

Background: Comprehensive lipidomics has allowed for unprecedented characterisation of the lipidome within biological systems. However, structural isomers (particularly phospholipid isomers) represent an ongoing challenge. Here we report on an improved targeted lipidomic methodology utilising UHPLC ESI-MS/MS with improved isomeric separation of lipid species, allowing for offline characterisation of structural isomers while maintaining high sensitivity and throughput. Method: Lipid species were extracted from 10µL of plasma with a single phase chloroform/methanol extraction. Extracts were examined by UHPLC ESI-MS/MS using an IPA/ACN/H2O solvent system and a non-linear gradient on a ZORBAX eclipse plus C18 column (2.1x100mm 1.8µm, rapid resolution high definition, Agilent) coupled with an Agilent 6490 triple quadrupole mass spectrometer. Characterisation of phospholipid species was performed using CID in negative ionisation mode and lithium adduct fragmentation in positive ionisation mode. Double bond positional isomers were characterised using synthesized standards. The method was used to examine a cohort of 347 plasma samples to assess assay performance and identify associations between lipid species and anthropometric measures (age and gender). Results: We identified over 600 lipid species including sphingomyelins with different sphingoid bases (d16:1, d18:0, d18:1, d18:2) and phospholipid isomers with different fatty acid compositions (i.e. 18:1/18:1 vs 18:0/18:2) or fatty acid isomers (i.e. 22:5 n3/n6). The lipidomic analysis identified sphingoid base specific associations with gender and long chain acyl carnitine with age. Conclusion: Our current lipidomic methodology enables the profiling of over 600 lipid species in 15 min from 10µL plasma and is suitable for high through-put analysis of large clinical cohorts.

O-449	Using U-13C glucose to determine the impairments in glucose metabolism in both acute and chronic epilepsy mouse models

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CO-AUTHORS: *Mark Hodson, Karin Borges*

There is growing evidence suggesting that metabolic disturbances contribute the development and progression of seizures. Here we aimed to elucidate which pathways in brain glucose metabolism are impaired in both the chronic "epileptic" stage of the pilocarpine-status epilepticus model and in the acute flurothyl model of epilepsy by tracing [U-13C]-glucose metabolism. Three weeks after pilocarpine-induced status epilepticus, mice were injected with [U-13C]-glucose (558mg/kg, i.p) and sacrificed 15 minutes later. In another cohort of animals, [U-13C]-glucose was administered either before or after mice experienced a 5 minute flurothyl-induced seizure. The percent enrichment of carbon-13 was measured in metabolites of the hippocampal formation using LCMS/MS and GCMS, and enzyme activities were measured via spectroscopy. Mice that develop status epilepticus had a reduction in the percent 13C enrichment in most TCA cycle intermediates (17-35%, p<0.05), coupled with 33% and 55% loss in the activities of pyruvate dehydrogenase and 2-oxoglutarate dehydrogenase, respectively (p<0.05 for both). Similarly, in the post-seizure stage of the flurothyl model the incorporation of 13C in the TCA metabolites was also reduced (46-93%, p<0.05), along with a 56% loss in pyruvate dehydrogenase activity. This loss of activity coincided with a 1.9-fold increase in phosphorylation of pyruvate dehydrogenase at ser232. Together this suggests that in epilepsy, impairments in pyruvate dehydrogenase activity reduce glucose entry into the TCA cycle. As the TCA cycle is critical for the production of ATP, which is essential to the recovery of ion and neurotransmitter balance, we have identified a promising target for anticonvulsant therapies.

O-466	Plasma metabolite profiles allow diagnosis and disease course monitoring of primary progressive multiple sclerosis

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Multiple sclerosis is considered to be an inflammatory and degenerative disease of the central nervous system (CNS). It shows high heterogeneity with regard to its pathology and clinical progression. Primary progressive multiple sclerosis (PPMS) is distinguished from relapsing-remitting multiple sclerosis (RRMS), by showing lower inflammatory activity with continuous neurodegeneration and rarely relapses. Here, we applied untargeted high-resolution metabolomics to identify plasma metabolites profiles that potentially allow diagnosis of PPMS and its differentiation from RRMS and healthy controls (HC). Moreover, we sought markers that significantly change during disease progression and could serve as surrogate markers of neurodegeneration over time. In an initial exploratory phase, plasma samples were analysed by untargeted metabolomics from a two independent sex- and age-matched PPMS and HC cohorts. Partial least square discriminant analysis (PLS-DA) yielded predictive models for robust within-cohort and residual cross-cohort classification along with as set of 20 informative metabolite markers which indicated significant alterations in glycerophospholipid metabolism in PPMS. Moreover, this metabolite signature showed to be PPMS-specific when compared against a sex- and age-matched cohort of another 10 HC and 10 RRMS patients. Finally, we investigated these metabolites in a longitudinal cohort of PPMS patients over 24 months (n = 15). Notably, the results indicate that the glycerophospholipid (PC(O-16:0/4:0)) significantly decreased during the observation period of PPMS (24 months). In summary, our study revealed PPMS specific metabolites, which show potential for non-invasive diagnosis and disease course monitoring of PPMS, and might serve as biomarkers for treatment efficacy in clinical trials of PPMS.

O-474	Systemic Impact of Roux-en-Y Gastric Bypass Surgery

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CO-AUTHORS: *Hutan Ashrafian, Florian Seyfried, Zhanxuan Wu, Qianxin Wu, Thanos Athanasiou, Elaine Holmes, Nigel Gooderham, Jeremy Nicholson, Julian Marchesi*

Obesity is a major health and socioeconomic problem worldwide. The most sustainable and effective treatment for morbid obesity is bariatric surgery such as Roux-en-Y gastric bypass (RYGB). Recent studies have shown that RYGB surgery induced systemic changes in gut hormones, energy metabolism, host-microbial co-metabolism and gut microbial composition in both human and rat models. We have applied multiple methods including metabolic, microbial and microRNA profiling to investigate systemic responses of the body to RYGB surgery. Through various in vivo and in vitro models, we have observed a microbial shift towards higher concentrations of Proteobacteria and lower concentrations of Firmicutes and Bacteroidetes in rodents after RYGB surgery. Decreased faecal tyrosine and increased putrescine suggested increased protein putrefaction. Increased urinary 4-cresyl compounds and decreased urinary TCA cycle intermediates indicate extensive disturbances in host-gut microbial crosstalk and host energy metabolism. Statistically significant changes of metabolites induced by RYGB were also observed in different sections of the intestinal tissues. These changes include amino acids, short chain fatty acids, choline-containing compounds and lipids, collectively suggesting that RYGB-induced metabolic changes may be associated with oxidative stress and membrane lipid metabolism. We also observed 14 significantly altered circulating miRNAs, suggesting modulation of signalling pathways including G protein signalling, neurodegeneration, inflammation, and growth and apoptosis responses. Our studies showed that RYGB surgery induced changes in both local and global metabolic activities. These findings aid our understanding of the metabolic phenotype of bariatric procedures and can facilitate development of alternative treatments for obesity-related diseases.

O-478	METABOLOMICS ANALYSIS DISTINGUISHES BETWEEN STAGES OF RENAL CELL CARCINOMA: A VALIDATION STUDY.

PRESENTING AUTHOR: *Oluyemi Falegan, University of Calgary, Canada*

CO-AUTHORS: *Andries Zijlstra, Hans Vogel, Eric Hyndman*

Renal cell carcinoma (RCC) is a heterogeneous disease and remains asymptomatic until late in the disease. RCC specific biomarkers that may be exploited clinically for diagnostic and prognostic purposes are therefore essential. We have previously shown that metabolomics and multivariate statistical analysis differentiate benign from malignant renal neoplasms. We sought to reproduce and validate our previous findings using a larger external cohort. Preoperative fasting urine samples were collected from patients with clinical renal masses and assessed with 1H NMR based metabolomics and multivariate statistical analysis. Alterations in the levels of carnitine, o-acetylcarnitine, gluconate, choline, tartrate, glycine, pyridoxine and methanol, and tricarboxylic acid cycle intermediates among other metabolites were detected in RCC relative to benign controls. Orthogonal Partial Least Square Discriminant Analysis plots discriminated between benign masses and pT1 (R2 = 0.44, Q2 = 0.37; AUC= 0.90), benign and pT2 (R2 = 0.52, Q2 = 0.36; AUC= 0.92), benign and pT3 (R2 = 0.331, Q2 = 0.233; AUC= 0.88) and benign and pT4 (R2 = 0.78, Q2 = 0.61; AUC= 0.90). The validity of all models was confirmed by negative permutations Q2 intercepts and CV-Anova p-values lower that 0.05. Our previous findings were largely reproduced in this validation cohort, NMR analysis of urine samples differentiated between benign lesions and stage 1, 2,3 and 4 malignant renal masses. Taken together these studies suggest that urine metabolomics may be useful in differentiating benign renal tumors from RCC and ultimately for staging and diagnosis of renal cell carcinoma.

O-511	Strategies for TCMs Q-Marker discovery based on Chinmedomics

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CO-AUTHORS:

TCMs Q-Marker is the parameters for evaluating the quality of TCMs, it must be contained in crude herbal drug, decoction pieces and Chinese patent medicine, it should be related with the clinical efficacy of TCMs and changed according to the compatibility of Chinese medical formula. It is difficult to discover the Q-marker from the thousands and tens of thousands of compound in single crude drug, moreover the combination of herbal medicines in formula, therefore a new strategy is in need. Chinmedomics is the integration of Serum pharmacochemistry of TCM with metabolomics to elucidate the scientific value of TCM, i.e, to discover the biomarker of TCM syndrome, to evaluate the efficacy of TCM formula, to analyze and identify the constituents in vivo originated from TCMs under the acting condition, and to mine the constituents in vivo which highly correlated with regulating of biomarkers of syndrome. By applying Chinmedomics strategy to discover the constituents related with the clinical efficacy of TCMs, used those constituents as Q-marker will express the inner quality of TCM.

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O-519	Does HIV/AIDS metabonomics disclose a general response to disease?

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CO-AUTHORS: *Aurelia Williams, Lungile Sitole*

Untargeted metabonomics using NMR, HRMAS-NMR, UPLC, IR- and Raman spectroscopic analysis of biofluids from HIV/AIDS patients revealed a similar metabolic fingerprint. Targeted GC-MS metabonomics analysis of HIV/AIDS-affected organic acids showed overlap with metabolites identified in the untargeted investigations. Associated with the latter study, cytokinomics revealed HIV-induced modifications of the immune response which coincided with organic acid metabolic data. In addition, oxidants and antioxidants were identified in both targeted and untargeted analyses, which is perhaps not surprising given the extensive evidence supporting HIV-induced oxidative stress as a general outcome of infection. Standard biochemistry was used to validate the metabonomic identification of glutathione following Raman spectroscopic analysis of HIV/AIDS biofluids. All the mostly untargeted investigations (HRMAS-NMR quantified 12 metabolites through HR-QUEST) referred to above did not present a specific metabolite or groups of metabolites that could be exclusively linked to HIV/AIDS, but the main pathways affected by the retroviral infection was confirmed. Chemometric tools to sort through the data abound, and there is also still the continuous addition of more statistical approaches, in this case, multinomial logistic regression, for successful sample classification. Quantification of more metabolites, targeted analysis of specific groups of metabolites (extractions directed at amino acids, lipid oxidation products or to detect sugars specifically, in each case using specific internal standards for referencing) and/or pooling data from different techniques to eliminate masking effects that overlapping metabolites cause, may support or refute a conclusion supported by current data that HIV/AIDS metabolomics discloses a general response to disease.

P-168	Metabolomic Identification of Diagnostic Biomarkers for Malignant Melanoma

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Previous metabolomics-based studies that distinguished melanoma from benign nevus relied on excision of lesions. There are no serum metabolomic biomarkers used for screening, staging, or optimizing treatment. Our objective was to utilize targeted metabolomic platforms to identify biomarkers for guiding diagnosis, staging workup, and surgical approach to melanoma patients. Twenty-six patients with stage III or IV melanoma and 46 controls were included. Serum samples were analyzed using nuclear magnetic resonance spectroscopy and liquid chromatography coupled with mass spectrometry. Data were analyzed using partial least squares-discriminant analysis. Stepwise logistic regression analyses were used to develop diagnostic algorithms in the training set (60%) and subsequently validated using an independent test set (40%). Areas under the ROC curves (AUC), sensitivity and specificity for diagnosis of melanoma were calculated. Topographical pathway analyses were performed to investigate metabolic dysregulations in melanoma patients. Targeted metabolomics significantly distinguished melanoma cases from controls (permutation test p-value=0.002). Five metabolites including PC aa C40:3, DL-carnitine, octanoyl-L-carnitine, ethanol, and methylmalonyl-L-carnitine were identified as optimal diagnostic biomarkers in the training group. The predictive algorithm was validated in the independent test group and achieved an AUC (95% CI) = 0.822 (0.665-0.979), sensitivity 70% and specificity 77%. Arginine and proline metabolism, tryptophan metabolism, glutamine and glutamate metabolism, glutathione metabolism and arginine and ornithine metabolism were significantly perturbed in melanoma patients (p<0.05). Targeted metabolomic analysis demonstrated significant differences in metabolic profiles of melanoma patients as compared to controls, representing exciting potential to develop serum-based risk stratification, predict prognosis, and direct systemic therapy and surgical management.

P-169	Untargeted serum metabolite profiling of colorectal cancer using GC-Orbitrap technology

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Globally affecting more than one million new persons each year, and killing more than 700,000, colorectal cancer is the second leading cause of cancer-related deaths in women and the third in men. Nevertheless, diagnosis is still largely based on invasive tissue sampling, while gaps remain in the understanding of its pathogenesis, with complex combinations between lifestyle, genetics, epigenetics, chronic inflammation (IBD) and microbiota. Untargeted metabolomics is one way to address these issues. Through metabolite profiling, it provides a picture of the outcome of the disease. To do so, significant variations between pathological and healthy phenotypes have to be found, and the responsible metabolites must be confidently identified. In this study, the ability of the Q Exactive GC-MS Orbitrap system to detect and identify metabolites related to colorectal cancer in an untargeted manner was assessed. The workflow uses the advantages of high peak capacity and chromatographic resolution of gas chromatography with the high resolution and sub-ppm mass accuracy of the Orbitrap mass spectrometer. The samples analyzed belonged to two populations linked to colorectal adenocarcinoma (active and remission, 12 samples each) along with two controls cohorts of the same size specifically matched for possible biases (gender, age, BMI, smoking status etc.), and pooled QC samples. Analytical raw data files were automatically processed through two software platforms specifically designed for the Orbitrap technology (TraceFinder™ and Compound Discoverer™). Compound identification was made using existing commercial libraries as well as an in-house developed high resolution Orbitrap metabolomics library.

P-170	Plasma metabonomics in patients receiving lung protective therapies during cardiopulmonary bypass: a comparative randomised controlled trial

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Introduction Lung injury following cardiac surgery with cardiopulmonary bypass (CPB) remains a challenge for physicians, with considerable associated morbidity and mortality. We recently described the metabolic response to standard CPB and the possible mechanisms of postoperative lung injury, however, there are no randomized controlled trials describing metabolic responses to lung protective therapies during CPB. Aim To validate previously identified alterations occurring during standard CPB and to examine how two protective pulmonary treatments influence plasma metabolite networks. Methods Nearly 800 plasma samples were collected from 90 patients randomised to standard CPB, or receiving either a pulmonary perfusion with oxygenated blood, or a hypothermic solution (histidine-tryptophan-ketoglutarate, HTK). Samples collected at six time points before (baseline) and after CPB were analysed by nuclear magnetic resonance spectroscopy and multivariate statistics. Results Similar metabolic perturbations occurred after CPB, validating our previous results. Increased levels of anaerobic metabolites, and decreased phospholipids, fatty acids, ketones, and several amino acids were detected in all patients, regardless of randomisation group. Patients receiving oxygenated blood had more profound changes in metabolites related to glycolysis, tyrosine, and arginine metabolism, while HTK patients demonstrated differences in glutamine-glutamate, glycine, alanine, creatine-creatinine, and purine metabolism, compared to the standard treatment group. Conclusion This study validates previous findings and shows metabonomics' potential in cardiac surgery and pharmacotherapy trials. While several metabolic differences were identified between groups, the identified networks need to be related to the inflammatory mechanisms known to elicit CPB-induced lung injury.

P-171	Metabolomics and qualitative flux analysis informed on the biology of response of AML to IACS-010759, a potent and selective OXPHOS inhibitor

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Acute myeloid leukemia (AML) is a highly aggressive and burdensome disease characterized by a high mortality rate among adults aged >65 years. Novel and more effective therapeutic interventions are therefore needed to improve the clinical outcome for this patient population. Our group has developed and characterized IACS-10759, a novel, potent and selective inhibitor of complex I of the mitochondrial electron transport chain leading to robust decrease in cell viability and increase in apoptosis across AML preclinical models and primary patient samples. We implemented mass spectrometry-based metabolomics and qualitative flux analysis to explore the mechanism of metabolic response of AML to IACS-010759 treatment. Targeted metabolomics revealed a drug-induced metabolic imbalance consisting of variations in amino acid and nucleotide production including ATP. Stable-isotope tracing revealed significant metabolic alteration consisting of a metabolic switch that resulted into an increased glycolytic rate leading to abnormal accumulation of lactate and alanine. This indicated the inability of AML to process pyruvate through the TCA cycle and thereby produce reducing power, notably NADH, for ATP production in the electron transport chain. Altogether these metabolic variations might account for the observed loss of cell viability and, in some cases, increased apoptosis. As a result of the robust response in multiple cell lines, primary patient samples, and efficacy in PDX models, IACS-010759 has been advanced through IND enabling studies and was approved by the FDA to start a Phase I clinical trial in AML patients which is currently undergoing at MD Anderson Cancer Center.

P-172	High Throughput Lipid Identification and Quantification Using a Directed HRAM LC-MS-MS approach on a Modified Quadrupole-Orbitrap Mass Spectrometer

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CO-AUTHORS: *David Peak, Andreas Huhmer*

Lipids play a key role in cell, tissue and organ physiology. In order to understand the biological function of lipids and identify unique lipid biomarkers for early disease detection, a robust and reproducible analytical method which enables large scale lipid identification and quantification over major lipid classes is required. Here we present that hundreds to thousands of individual lipids can be identified and quantified from any biological complex samples using a HRAM LC-MS-MS approach which implements a very large pre-defined lipid precursor ion inclusion list for directed MS-MS data acquisition on a modified quadrupole-Orbitrap mass spectrometer. Lipid extracts from different type of samples (human plasma, bovine liver, bovine heart) were used. An isotopic labeled lipid mixture with known concentration was spiked into each sample before LC-MS-MS run. A C30 column was used for lipid separation and a modified designed quadrupole Orbitrap mass spectrometer was used for lipid detection. For data acquisition, a HRAM full MS scan was followed by up to 30 HRAM MS-MS scans which would be triggered only when the detected precursor ions from survey MS scan match the lipid inclusion list. The lipid identification and simultaneous quantitation were performed using LipidSearch software. The absolute amount for each identified lipid species was estimated using the isotope-labeled lipid standard which belongs to the same lipid class as the identified lipid species. Excellent analytical precision was observed using this method and the CV was less than 15% for internal standards and most of the lipid species quantified.

P-173	Mass Spectrometry Glycosaminoglycan Analysis for Mucopolysaccharidoses: a Reliable and Non-Invasive Tool for Detection, Diagnosis and Follow Up of Patients

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CO-AUTHORS: *Pamela Lavoie*

Mucopolysaccharidoses (MPS) are lysosomal storage disorders leading to glycosaminoglycan (GAG) accumulation. Our project aimed to: 1) analyze a wider spectrum of urinary GAGs to improve the detection, diagnosis and monitoring of patients affected with various types of MPSs (MPS I, II, III, IV, VI, VII); 2) develop/validate a quantitative multiplex mass spectrometry (MS) method for the urinary analysis of dermatan sulfate, heparan sulfate, keratan sulfate, and chondroitin sulfate disaccharides; 3) compare this MS method with a spectrophotometric method used in many clinical laboratories. A methanolysis reaction was performed by adding methanol-HCl·3N to a dried urine sample, followed by heating at for one hour, and nitrogen evaporation. The residue was dissolved in 200 mL of a solution containing synthesized in-house deuterated internal standards, followed by injection onto the Acquity I-Class/ Xevo TQ-S MS/MS system (Waters Corp.) in positive electrospray. Intraday precision assays were good (RSDs ? 7.6%), as well as accuracy results with biases from -12.0% to 8.4%. Interday precision and accuracy assays showed RSDs ? 8.7 % and biases from -12.8% to 14.2%. This 7-min method offers high resolution/specificity to differentiate different types of MPS patients. In comparison to the MS/MS method, the spectrophotometric assay showed that 30% of MPS patients from our cohort had normal results and 13% had borderline results, thus confirming its unreliability. This efficient UPLC-MS/MS method allows the absolute quantification of four GAGs for six MPS types. A GAG profile was established for diagnostic purposes, monitoring and follow up of patients, and evaluation of treatment efficacy.

P-174	Genetic and metabolomic analyses of the de novo L-cysteine biosynthetic pathway in the enteric protozoan parasite Entamoeba histolytica

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Cysteine biosynthetic pathway is of fundamental importance for the growth, survival, antioxidative stress defense and pathogenicity of many pathogens such as Entamoeba histolytica, Trichomonas vaginalis, Leishmania major and Salmonella typhimurium. The enteric protozoan parasite E. histolytica is completely devoid of glutathione and its metabolism, and cysteine is the principal low molecular weight thiol. The de novo cysteine biosynthetic pathway in E. histolytica involves two key enzymes, serine acetyltransferase (SAT) and cysteine synthase (CS), which is absent in mammals therefore, considered as promising targets for inhibiting the growth of this parasite. E. histolytica apparently possesses three SAT (EhSAT1-3) and three CS (EhCS1-3) isozymes. In order to fully understand the role of this pathway we have created ameba cell line in which expression of the genes involved in cysteine biosynthesis pathway is completely knocked down. Using metabolomics approach we found that this pathway is involved in S-methylcysteine synthesis. CS and SAT3 knockdown parasites showed impaired growth when cultured in cysteine lacking media while SAT1/2 doesn`t, suggesting importance of these enzymes in parasite biology and therefore could be exploited as drugs target against this pathogen. Further to discover inhibitors against the EhCS the compounds of Kitasato Natural products library were screened against two recombinant CS isozymes: EhCS1 and EhCS3. Nine compounds inhibited EhCS1 and EhCS3 with the IC50 values of 0.31-490 microM. Since CS is also present in other protozoan, the newly identified CS inhibitors in this study can also be exploited to develop drugs against other neglected parasitic diseases.

P-175	Complementary metabolic profiling reveals new insights into fatty liver disease and associated comorbidities among healthy individuals

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Triglyceride accumulation in the liver, so-called fatty liver disease (FLD) represents a risk factor for metabolic diseases like type 2 diabetes (T2DM) or cardiovascular diseases (CVD). Despite the recognition of impaired insulin signaling the molecular mechanisms involved are still poorly understood. We combined MS and NMR techniques to comprehensively characterize plasma and urine samples from 762 non-diabetic participants of a population-based sample. Liver fat content (LFC) was assessed using quantitative chemical shift encoded MRI. Associations between LFC as well as markers of hepatic damage and the metabolome were assessed by multivariable linear regression analyses controlling for several confounders. An integrated multi-fluid metabolite analysis was facilitated by the use of Gaussian graphical modelling (GGM). A predictive molecular signature of FLD was established using the LASSO embedded in a two-stage cross-validation procedure. As expected alterations in lipoprotein (atherogenic profile) and fatty acid metabolism were pronounced and strongly interrelated, whereby associations to free fatty acids were unique to liver enzyme activities. GGM analyses revealed an enriched cluster of metabolites uniquely associated with LFC, comprising branch-chained and aromatic amino acids. Even associations to urine metabolites were almost unique to LFC. In particular, a part of this urine signature improved the predictive performance for FLD moderately in comparison to classical factors. The application of untargeted metabolomics revealed a metabolic fingerprint of LFC which mimics molecular profiles associated with the progression of T2DM and CVD. Moreover, the comprehensive metabolic profiling applied here allowed for systemic understanding of previously separately presented molecular events associated with liver disease.

P-176	How diets modulate the metabolome: lessons from MyNewGut

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In 2013 the MyNewGut consortium brought together 30 partners to provide practical solutions to reduce the obesity epidemic that affects numerous countries. Here we will present the results of three studies that aimed to evaluate the metabolic impact of common diets in humans and animal models using a NMR-based metabolomics approach. 1) The impact of high fat diet on brain metabolism was studied in mice. The metabolic profile of the hypothalamus, hippocampus, striatum and prefrontal cortex regions revealed that high-fat diet might be responsible for a metabolic shift. Specifically, an increase of energy metabolism with higher levels of lactate and creatine phosphate in the prefrontal cortex were detected. 2) In a randomised cross-over human study, AXOS (arabinoxylan-oligosaccharides) prebiotic or high PUFA (polyunsaturated fat acid) diet was given to obese/overweight subjects with metabolic syndrome to asses the effect of diet on energy balance and metabolic biomarkers. Preliminary results confirmed that PUFA tended to reduce plasma triglyceride levels but this was susceptible to high inter-individual variability. 3) A human intervention studying high protein diet, which is generally perceived by the public as healthy, showed changes in individual metabolomic profiles associated with the disfavoured decrease of butyrate, a metabolite known to improve gut health. These data demonstrate that diet composition has far reaching metabolic consequences that can vary hugely from one individual to another due to individual phenotypes. These data complement the increasing awareness of the unsuitability of the one-size-fits-all diet recommendation and encourage a future personalised approach to nutrition guidelines.

P-177	Metabolic phenotyping to assess the system-wide impact of pediatric critical illness on gut health

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CO-AUTHORS: *Sarah Thurston, Adilson Sabino, Sara Zaher, Debbie White, Jenna Ridout, Elaine Holmes, Nazima Pathan*

Gut dysfunction has been shown to occur frequently in intensive care patients. In critical illness, damage to the intestinal mucosa can occur, compromising the epithelial barrier which functions to separate gut microbes and toxic products from the host system. When bacterial antigens translocate from the intestinal lumen into the bloodstream or lymphatic system, sepsis or multi-organ failure can result. Studies in critically ill adults have shown that the loss of normal intestinal microbial species and a reduction in beneficial bacterial co-metabolites such as butyrate (a short chain fatty acid and key energy source for colonocytes), is associated with disease severity and outcome. We applied a combined 1H-Nuclear Magnetic Resonance Spectroscopy and Mass Spectrometry approach coupled to multivariate statistical analysis, to capture the system-wide impact of critical illness, in a cohort of pediatric intensive care patients. Results reveal differences in metabolic phenotypes between patients compared to age-matched controls, including markers of gut health (microbial co-metabolites and short chain fatty acids), branched chain fatty acids, lactate and several amino acids. Since urine and stool phenotypes carry information on microbial co-metabolites as well as chemical signals relating to metabolic end products, the acquired spectroscopic data provides functional assessment of the microbiota as well as aiding in understanding the system-level molecular pathways that are altered in critical illness. The ultimate goal of this research is to identify pathways by which to target early nutritional intervention to treat gut dysfunction and improve gut health, and thereby improve short and long-term outcomes of pediatric critical illness.

P-178	Robust and High Throughput Lipid Profiling of dry blood spot samples using Automated FIA MSMSAll technique

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Lipidomics is opening new ways to clinical research though it is in a nascent stage. One of the major challenges in the study of lipids and other biological molecules is the analysis time. We have developed a high throughput and robust method on TripleTOF® 5600 system using Dried Blood Spot (DBS) samples during clinical research, so as to simplify overall procedure and shorten the assay time. Majority of the literature so far is based on using either plasma or serum considered as gold-standard matrix but both these media require a phlebotomist, functional laboratory and storage at very low temperatures - 40oC to -70oC. Advantage of using DBS over the plasma/serum makes it ideal for screening studies. 90 dried blood spot samples were used for the analysis. The lipid extracts were analyzed using Shimadzu LC XR system coupled to the TripleTOF® 5600 system. Samples were analysed using the Infusion MS/MSALL workflow for complete lipidome coverage. The MS/MSALL workflow experiments performed the data independent acquisition and consisted of a TOF MS scan followed by a sequential acquisition of 1001 MS/MS spectra acquired at a step size of 1 Da. The lipid identification was performed by the LipidView™ software. Batch processing for lipid identification was performed for all the replicates samples. In this shotgun approach we had been successfully able to get coverage of about 1031 lipids in all detected classes. Lipid classes like DAG, NAPE, PC, CE, PE, TAG were found to be abundantly in the DBS samples.

P-179	Metabolic phenotyping of formalin-fixed, paraffin-embedded colorectal cancer tissue samples by DESI-MSI

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CO-AUTHORS: *Renata Soares, Luisa Doria, James McKenzie, Francesca Rosini, Zoltan Takats*

Desorption electrospray ionisation mass spectrometry imaging (DESI-MSI) is a powerful technique which creates ion images from mass spectra collected for tissue samples. These images are then used to show the spatial distribution of biochemical information over the sample area and can contribute to diagnosis of various diseases. This approach has been successfully employed in fresh frozen tissue samples, however since formalin-fixed, paraffin-embedded (FFPE) clinical tissue samples are the standard for histopathological analysis, much effort has been spent on optimising and standardizing FFPE protocols for DESI-MSI. In this particular study tissue microarrays (TMAs) were used. TMA contains many small representative tissue samples from hundreds of different cases assembled on a single glass slide, and therefore allows high-throughput analysis of multiple specimens at the same time. 10µm thick tissue sections were cut from a TMA block composed of samples from 18 patients (malignant tumour, cancer and normal adjacent tissue cores). The tissue sections were analysed in negative ionization mode by DESI-MSI, stained with H&E, annotated and then aligned with ion images for regions of interest. Metabolic information remaining in the tissue cores after standard histological FFPE processing allowed the discrimination between different tissue types in multiple samples (overall accuracy over 95%). m/z peaks were assigned to tissue types (e.g. m/z 599.3 tumour, m/z 314.1 and m/z 350.1 both cancer and normal adjacent tissue) and their identification was confirmed using LC-MS. This approach could help to reveal novel set of species and therefore contribute to the understanding of metabolism.

P-180	Targeted and Non-targeted Quantitative Metabolomics Using a Multi-platform Approach

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TMIC (The Metabolomics Innovation Centre) is Canada's national metabolomics laboratory specializing in quantitative metabolomics assays for human, animal, plant and microbial samples. TMIC provides low-cost services to academia and industry, develops/maintains freely available metabolomics databases and web servers (HMDB, DrugBank, T3DB, CFM-ID, FooDB, MetaboAnalyst) and develops innovative assays. TMIC provides: 1) software and databases for data interpretation and analysis; 2) platform-specific, fully quantitative metabolomic assays; 3) untargeted, semi-quantitative metabolomic assays; 4) pathway-specific assays; 5) class-specific assays; 6) customized assays (based on client requests); 7) the Human Metabolome Library (a chemical library consisting of >1130 metabolites); 8) metabolomic profiling kits; 9) bioinformatics and statistical support; and 10) specialized services (programming, experimental design) to support a wide range of metabolomics applications and projects. TMIC's technologies are based on NMR and mass spectrometry. TMIC is constantly working towards developing, acquiring, testing and implementing new, quantitative metabolomic technologies. Most recently, TMIC has developed and adapted several quantitative assays to expand the list of detectable metabolites to include catecholamines, steroids, oxylipins, one-carbon metabolites, organic acids and volatiles. In addition, TMIC has incorporated two additional Canadian laboratories (TMIC nodes) adding new capabilities and resources to TMIC's network. TMIC has also developed a series of quantitative metabolomics kits consisting of reagents, protocols and/or software that can simultaneously, inexpensively and quantitatively measure a large number of metabolites in human biofluids. TMIC's new assays and technologies along with a brief description of their applications in human health, agriculture, nutrition and other fields are presented in this poster.

P-181	A comprehensive metabolomic profiling of blister fluid from paediatric burn patients

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Burn injury is a common and highly traumatic event in paediatric populations. At present, the diagnosis of burn injury severity is largely dependent on the clinician's experience. A better understanding of burn injury biochemistry would facilitate the development of quantitative measures to aid diagnosis. Burn blister fluid (BF) is a viable source of biomolecules that reflect relevant systemic responses and the local microenvironment. Metabolomics is a powerful technique for the discovery of novel biomarkers and elucidation of biochemical pathways to assist with clinical diagnosis. Nuclear magnetic resonance (NMR) spectroscopy (Bruker 600MHz) data were utilised for multi-parametric metabolic quantification analysis of BF from paediatric burn patients in our investigation. One hundred and one BF samples with different clinical variables, such as burn depth, burn size, and time to re-epithelialisation, were recruited. The fluid samples were collected by syringe during routine blister de-roofing procedures. The measurement parameters and sample buffer were optimised based on the NMR serum protocols. Proton NMR spectra were phased, baseline corrected, and referenced to sodium formate using TopSpin software (Bruker). Spectra were bucketed (AMIX software, Bruker) and the buckets profiled using an R package, BATMAN, to perform Bayesian deconvolution and quantification of metabolites. All of the 757 metabolites from the database build in BATMAN were quantitatively profiled. The statistical analysis of the metabolomics of burn BF was completed through MetaboAnalyst. Significant metabolites between different burn depth and time to re-epithelialisation were discovered. In addition, pathways related to burn injury and wound healing were revealed.

P-182	Molecular Transducers of Physical Activity Consortium (MoTrPAC): Creating a Comprehensive Map of Molecular Changes in Response to Physical Activity

PRESENTING AUTHOR: *Padma Maruvada, National Institute of Diabetes, and Digestive and Kidney Diseases, United States*

CO-AUTHORS: *Maren Laughlin, Ashley Xia*

The NIH Common Fund established the Molecular Transducers of Physical Activity Consortium (MoTrPAC) in 2016 by issuing 19 grants to 37 Principal Investigators from 23 institutions. Aim 1: MoTrPAC will assemble a comprehensive map of the molecular changes that occur in response to exercise and provide insights into how they are altered by age, sex, body composition, and fitness level. Aim 2: To fulfill its overall objective of facilitating investigator-initiated studies and catalyzing the field of physical activity research, MoTrPAC will develop a user-friendly database, accessible to researchers for pursuing research on the benefits of physical activity. Approach: MoTrPAC Clinical Center investigators will recruit approximately 3,000 healthy subjects across the lifespan for an exercise study carried out at twelve sites across the United States. A variety of specimens namely blood, muscle and adipose tissues will be collected from study participants. In addition, research teams at three Preclinical Animal Study Sites will use a rat model to complement the clinical work. Samples will be analyzed by eight MoTrPAC Chemical Analysis Sites using extensive –omics analyses. In parallel with these efforts, the Bioinformatics Center will provide analytic tools for integrating and interrogating all of the MoTrPAC data into a searchable, publicly available database. This large project will be supported by a Consortium Coordinating Center and overseen by a Steering Committee, including the NIH. Our presentation will provide an overview of the MoTrPAC conosritum and approaches to develop a map of molecular signals in response to physical activity in humans. https://commonfund.nih.gov/MolecularTransducers/overview

P-183	Optimisation and investigation of seminal plasma for non-invasive prostate cancer diagnosis and monitoring: a NMR-based metabolomics study

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CO-AUTHORS: *Renee Richards, Clement Chow, Marion Buck, John Yaxley, Martin Lavin, Robert Gardiner, Horst Schirra*

Background: Non-invasive biomarkers are required to improve prostate cancer (PCa) care. NMR-based seminal plasma metabolomics may be a suitable platform. Sample collection is complicated by enzymatic (prostatic acid phosphatase; PAP) influence on metabolite levels. Methods: 151 seminal plasma samples from men "at risk" of prostate cancer were analysed using 1H-NMR spectroscopy. Buffer (Add-to-Subtract) and endogenous enzyme metabolite adjustment was performed prior to metabolite profiling with multivariate statistical analysis (principal components analysis, partial least squares) and targeted quantitation. In an independent cohort, PAP was inhibited using tartrate and the subsequent effects on the kinetics of phosphorylcholine to choline hydrolysis were determined. Results: Metabolites in seminal plasma best distinguished low- and intermediate-risk prostate cancer from benign samples. High grade samples were dominated by lipids/lipoproteins with less metabolite contributions overall. Previously described metabolites were not validated for prostate cancer prediction. Tartrate in increasing concentrations and/or refrigerated sample storage (279 K) reduced apparent rate constants, indicating greatly reduced reaction rates. Metabolite profile multivariate analysis showed that tartrate addition stabilises phosphorylcholine and choline concentrations while maintaining personal differences in metabolite profiles. Conclusion: Seminal plasma metabolomics in vitro may assist clinicians to more accurately diagnose or monitor either low or intermediate grade prostate cancer. Less clinical benefit may be observed for high-risk patients. Sample collection has been optimized and should be implanted in seminal fluid metabolomics studies. Further investigation in active surveillance cohorts, and/or in combination with in vivo magnetic resonance spectroscopic imaging may further optimize localized prostate cancer outcomes.

P-184	Untargeted metabolite profiling of maternal urine, serum, and hair successfully identified biomarkers of dietary patterns related to GDM development

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Background: Gestational diabetes mellitus (GDM) is glucose intolerance that is first recognised during pregnancy. Maternal diet may play an important role in the development of GDM. Although food frequency questionnaires (FFQ) are often used to characterise dietary patterns, these self-reported assessments are subject to recall and social desirability biases. Therefore, the aim of this study was to combine dietary pattern and metabolomic data to validate the associations between dietary intake and GDM from a metabolic perspective. Method: A total of 50 GDM cases and 50 controls were recruited at 26–28 weeks of gestation. Dietary intake was assessed using a 96-item FFQ. Clinical characteristics were obtained from medical records. The urine, serum, and hair metabolomes were analysed using gas chromatography-mass spectrometry and liquid chromatography-mass spectrometry. Results: Two maternal dietary patterns were significantly different between the GDM and control groups: High Glycemic Index (HGI) pattern and Low Glycemic Index (LGI) pattern. The HGI pattern was associated with an increased likelihood of GDM (OR: 2.13; 95% CI: 1.16, 3.29) and LGI pattern was associated with a decreased likelihood of GDM (OR: 0.56; 95% CI: 0.33, 0.94). A set of 28 and 70 metabolites differentiated GDM cases from controls using GC-MS and LC-MS, respectively. Of the 98 significant metabolites, 10 were significantly associated with the HGI pattern, and 17 were significantly associated with the LGI pattern. Conclusions: Metabolomics adds significant value to dietary investigations as it can assist with the elucidation of metabolic mechanisms underpinning the associations between the maternal diet and GDM development.

P-185	Metabolic profiling of polychlorinated biphenyls(PCBs) and organochlorine pesticides(OCPs) exposure in human plasma

PRESENTING AUTHOR: *Seoyoung Jang, Korea Basic Science Institute, South Korea*

CO-AUTHORS: *Geum-Sook Hwang*

Polychlorinated biphenyls(PCBs) and organochlorine pesticides(OCPs) are persistent organic pollutants (POPs) known as environmental toxins. Accumulation of POPs has adverse effects on both environment and human. However, the association between POPs and human metabolism are unknown. To observe the effect by accumulation of POPs in humans, we investigated the associations between POPs levels and concentrations of metabolite detected in human plasma samples. In this study, concentrations of total 33 POPs including 16 PCBs and 17 OCPs were analyzed from plasma samples of 300 participates without occupational exposure to POPs. PCB118, 138, 153, 180, and 187 contribute to 85% to total PCBs, and dominant OCPs were HCB, ?-HCH, p,p'-DDT, p,p'-DDE, trans-nonachlor, and cis-nonachlor. The sum of the signed ranks of five PCBs and six OCPs, respectively, were used in regression analysis. Then, we conducted plasma metabolomic profiling using 1H-NMR spectroscopy. 22 metabolites were qualified and quantified from human plasma samples. We found that PCBs were significantly correlated with creatine, glucose, mannose, citrate, and phenylalanine. On the other hands, OCPs were associated with creatine, mannose, lactate, and phenylalanine. This study has shown that the NMR-based metabolic approach can be useful tool to understand the alteration of human metabolism and to identify potential biomarkers for estimating exposure effect of environmental toxins like POPs.

P-186	Lipidomic Study of Environmental Exposure: Effects of Perfluoroalkyl Substances and Phthalates on Children

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CO-AUTHORS: *Zhi-Yi Du, Guang-Wen Lien, Wan-Yu Lin, Pau-Chung Chen, Ching-Yu Lin*

Perfluoroalkyl substances (PFASs) and phthalates are two groups of chemicals which are commonly used in man-made products, therefore can be detected in human generally. Recent epidemiologic surveys which focus on PFASs and phthalates still cannot provide strong association between the exposure and the adverse health effects and suggest possible mechanisms. Numerous animal studies showed that these chemicals disrupt the endocrine system and might influence the regulation of lipid metabolism and fatty acid storage. Since lipids are possible target molecules of PFASs and phthalates, the purpose of this study is to identify changes of critical lipids caused by those chemical exposure and to understand possible biological impacts. 290 Taiwanese children exposed to environmental PFASs and phthalates were included. 13 PFASs and 12 phthalate metabolites were analyzed in their biofluids by HPLC-MS/MS. Two major lipid groups, phosphatidylcholine and sphingomyelin, in serum samples were analyzed by UPLC-MS/MS followed by multivariate statistical analysis to examine the differences of serum lipdome in children exposed to different levels of PFASs and phthalates. The results showed children exposed to different levels of perfluoro-n-tridecanoic acid, perfluoro-n-decanoic acid and di(2-ethylhexyl) phthalate had distinct lipidomes. Futhermore, the lipidomes of children's serum were associated with genders, body mass indexes and residential regions. Lipids associated with PFAS and phthalate exposures and other factors were identified and linked with possible mechanisms and adverse effects. This study demonstrated MS-based lipidomic approaches are powerful tool to realize lipid perturbation caused by background levels of PFASs and phthalates and to suggest possible adverse health effects..

P-187	Metabolomics application in discovery of novel mechanisms during insulin stimulated glucose uptake

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CO-AUTHORS: *Solvejg Hansen, Bente Kiens, Jonas Treebak*

It is well known that insulin increases glucose uptake. However, the underlying mechanisms have not been fully clarified. We speculate that the analysis of plasma metabolome during insulin stimulation can uncover novel pathway involved in glucose levels regulation. As part of the Copenhagen Women Study lean, healthy females (n=9; BMI<25kg/m2) were recruited for the study. The volunteers arrived in the morning after an over-night fast. After resting in the supine position for 1 hour, a hyperinsulinemic euglycemic clamp was initiated (insulin infusion rate 1mU/kg/min) and performed for 2 hours. Before the clamp and during the steady state of glucose infusion (80 min) EDTA plasma samples were collected. Insulin levels at the baseline were 5.8±1.8 µU/mL. After 80 min of insulin infusion the levels averaged 80.8±7.8 µU/mL. Following methanol extraction, plasma metabolites were profiled using UHPLC (Dionex Ultimate 3000, Thermo Scientific) with a Luna Polar C18 column (1.6 µm, 2.1x100 mm, Phenomenex). LC was coupled with QToF mass spectrometer (Impact II, Bruker Daltonics) operating in electrospray ionization. XCMS and CAMERA were used for metabolic features extraction and annotation, resulting in approximately 20,000 buckets in positive and negative mode combined. MetaboAnalyst was used for statistical analysis. PCA score plot showed a clear separation between baseline and clamp. Plasma metabolome was significantly altered during insulin infusion affecting not only glucose, but hundreds of metabolites when compared to the baseline (p<0.05, FDR<0.1). Further MSMS analysis is required to identify the significantly shifted metabolites and relevant pathways for insulin-stimulated glucose uptake.

P-188	Lipid metabolism of ovarian cancer – novel perspectives for diagnostics and therapy by DESI-MSI

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Ovarian cancer is the most fatal female reproductive cancer worldwide and there is an increasing demand for the development of ovarian cancer diagnostics with improved performance. Lipids are a class of metabolites with great potential to characterize and uncover changes in cancer lipid metabolism, which is still poorly understood. Different techniques were therefore combined to better understand lipidomic variations in epithelial ovarian carcinoma (EOC). The lipidomic profile and spatial information of over 100 EOC samples were obtained by DESI-MSI (desorption electrospray mass spectrometry imaging), validated by LC-MS and correlated with immunohistochemistry results of different enzymes related to lipid metabolism (phospholipid and fatty acid pathways). From the vast number of the hallmarks of cancer already studied, it has been shown that lipids play a crucial role in most of them. For example, our results show an increase of the phosphatidic acid class (PA) as well as its products from the CDP-DAG pathway (Kennedy pathway); phosphatidylinositol (PI) and phosphatidylglycerol (PG). From the immunohistochemistry performed in the enzymes around this pathway, PLD showed a substantial increase in its expression, interestingly linked to mTOR expression, which is essential for cancer survival. Further changes were observed and validated with LC-MS in the different phospholipid classes and its fatty acid chain length composition. Overall, DESI-MSI was an excellent technique to identify the lipidomic profile and changes in ovarian cancer, with robust correlation by our immunohistochemistry results, showing the potential of DESI-MSI to complement histopathology at a diagnosis and potentially therapy level.

P-189	Tandem Mass Spectrometry Analysis of Gb3 Isoforms and Analogues in Different NOD/SCID Fabry Mice Tissues

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CO-AUTHORS: *Philippe Provençal, Jeffrey Medin, Christiane Auray-Blais*

Fabry disease is an X-linked lysosomal storage disorder caused by a deficiency in alpha-galactosidase A activity, leading to storage of glycosphingolipids, such as globotriaosylceramide (Gb3), in tissues and biological fluids. It is characterized by cardiac, renal and neurological involvement. Recent metabolomic studies performed in our laboratory revealed 22 different Gb3 isoforms and analogues as Fabry disease biomarkers. The objective of this study was to evaluate the levels of these biomarkers in different organs (kidney, brain, liver, heart, lung, small intestine, spleen), and in urine and plasma from nonobese diabetic (NOD)/severe combined immune deficiency (SCID)/Fabry mice (9 males and 9 females) and from NOD/SCID control mice (3 males and 2 females). These strains allow for a matched genetic background and a reduction in systemic lymphocytes. Tissue samples were homogenized in methanol using a bead mill. Tissue homogenates, urine and plasma were purified by extraction with tert-butyl methyl ether. The organic phase was separated by ultra-performance liquid chromatography (Acquity I-Class, Waters) and analyzed by tandem mass spectrometry (Xevo TQ-S, Waters) using the multiple reaction monitoring mode in positive electrospray. As expected, biomarker levels were higher for Fabry mice compared to controls. Surprisingly, total Gb3 levels were significantly higher in the spleen and small intestine tissues compared to kidney and heart tissues, the latter organs being involved in Fabry patients' premature death/morbidity. The method developed and validated for this study will be used to evaluate the clearance of Gb3 isoforms and analogues in the first gene therapy clinical trial for Fabry patients.

P-190	Elucidation of the mechanisms of rice bran supplementation on the suppression of colitis development

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CO-AUTHORS: *Wanping Aw, Masaru Tomita, Shinji Fukuda*

Oryza sativa, rice bran (RB) has reported colitis suppression effects, but the underlying mechanisms are not yet understood. We aimed to elucidate the molecular mechanisms of RB dietary intervention on a 2.0% dextran sulfate sodium (DSS)-induced colitis C57BL/6J mice model. Body weight loss, elevated disease activity index, reduced colon length and colon histopathology were improved in RB-fed mice. While many studies have shown that gut microbiota is altered by DSS administration, these studies only report a few time points in their analysis. In this study, we took a novel approach by investigating fecal microbiota and fecal metabolites via comprehensive microbiome (Miseq Illumina, Inc.) and metabolome (capillary electrophoresis with electrospray ionization time-of-flight mass spectrometry (CE-TOFMS), Agilent, Inc. and gas chromatography mass spectrometry (GC-MS), Agilent, Inc.) approaches from before to 10 days after DSS administration every single day to elucidate the intestinal dynamics, and identified the RB-related gut environmental alterations in DSS-induced colitis mice. 26 microbial families, especially Alcaligenaceae and Lactobacillaceae showed significant increases and Enterobacteriaceae and Erysipelotrichaceae were significantly decreased in RB-fed mice. 60 metabolites, especially glutarate, 4-pyridoxate, N1-acetylspermidine, 5-hydroxy-indoleacetate and short-chain fatty acids were significantly increased in RB-fed mice. Metabolite set enrichment analysis revealed that Vitamin B6 metabolism, tryptophan metabolism and ubiquinone biosynthesis were significantly increased in RB-fed mice. These results suggest that RB-related alterations of gut environment have the potential to prevent colitis through maintaining a robust gut environment. Our multi-omic approach and findings provide an important tool in developing new nutritional therapeutic applications of RB in colitis prevention.

P-191	Dysregulated Lipid Profiles of Non-Alcoholic Fatty Liver Disease (NAFLD)

PRESENTING AUTHOR: *James Broadbent, SCIEX, Australia*

CO-AUTHORS: *Timothy Garrett, Kenneth Cusi, Baljit Ubhi*

Nonalcoholic fatty liver disease (NAFLD) is an umbrella term for a range of liver conditions affecting people who drink little to no alcohol. Main characteristics of nonalcoholic fatty liver disease is too much fat stored in liver cells. Nonalcoholic steatohepatitis, a potentially serious form of the disease, is marked by liver inflammation, which may progress to scarring and irreversible damage. This damage is similar to the damage caused by heavy alcohol use. At its most severe, nonalcoholic steatohepatitis can progress to cirrhosis and liver failure. However, if caught early the fatty liver and fatty liver to NASH process can be reversed. The Lipidyzer™ platform was used to evaluate two stages of NAFLD, to evaluate any dysregulation in complex lipid metabolism. Quantitative profiling of over 1100 lipid species from 13 different lipid classes was performed providing extensive lipid coverage. Quantitative lipidomics was used to profile the changes in complex lipid metabolism that occur across the stages of NAFLD, using human plasma samples from 3 known disease groups. The platform quantitated 851 lipid molecular species covering ceramides, cholesterol esters, lyosphosphopholipids, phospholipids, neutral lipids (TG and DG), and free fatty acids across the sample set analyzed with good reproducibility. There were a number of lipid species that had statistically significant changes from control as the disease progressed from simple steatohepatitis to steatohepatitis. The top 10 up-regulated and down-regulated lipid molecular species mainly consisted of cholesteryl esters and triacylglycerides.

P-193	Characterization of aberrant lipid metabolism in human esophageal cancer by lipidomic profiling

PRESENTING AUTHOR: *Yanjun Hong, Hong Kong Baptist University, Hong Kong*

CO-AUTHORS: *Zhiyi Yang, Zhongjian Chen, Xiaojiao Yi, Zhenyu Guo, Wenhao Ji, Zongwei Cai*

Esophageal cancer (EC) is the sixth leading cause of cancer-related mortality and the eighth most common cancer worldwide. Metabolic reprogramming, including lipid metabolism dysregulation, has been acknowledged as one of the hallmarks of cancer cells. Previous studies have shown the perturbations of several lipids in EC patients. However, due to limitations in technology, only a few lipid species were analyzed in those studies. Lipidomic profiling, which enables characterization of lipid structures and compositions, has been widely applied in cancer research to study tumorigenesis and identify potential lipid biomarkers. In this study, to further characterize the aberrant lipid metabolism in EC, liquid chromatography—tandem mass spectrometry (LC-MS/MS) based lipidomics were performed on paired tumor and adjacent normal tissues from 85 EC patients. Distinct alterations of global lipid metabolism were indicated in EC tumor tissues based on the partial least-square discriminative analysis (PLS-DA) model (R2Y=0.797 and Q2Y=0.751 in positive mode; R2Y=0.702 and Q2Y=0.673 in negative mode). Over 100 potential lipid biomarkers were found to be perturbed in EC tumor tissues, with phosphatidylcholines (PCs) and phosphatidylethanolamines (PEs) being mostly elevated. Upregulated phospholipid biosynthesis was found in EC tumor tissues, indicating the requirements of continuous lipid biosynthesis for proliferation by cancer cells. Furthermore, the abundances of medium-chain triglycerides (TGs) were decreased, suggesting immune-suppression effects in EC. Collectively, our results unravel the major lipid metabolic aberrations in EC tumor tissues for the first time, and imply the potential of lipid biomarkers for EC diagnosis.

P-195	Environmental pollutant BDE 47 enhances adipocyte differentiation via an elevation of oxidative stress

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CO-AUTHORS: *Chi-Ming WONG, Juntong WEI, C.K. CHUNG, Zongwei CAI*

Recently, obesity is dramatically increased globally and the conventional causes cannot fully explain its rising prevalence. Emerging evidence suggests the contribution of persistent organic pollutants (POPs) to the prevalence of obesity. Clinical studies demonstrated the association of 2,2',4,4'-tetra-brominated biphenyl ether (BDE 47) with obesity but the mechanism is still unclear. We examined the effect of BDE 47 exposure during 3T3-L1 preadipocyte differentiation and explored the metabolic alterations via metabolomics study using liquid chromatography coupled with mass spectrometry (LC-MS). Results indicated that BDE 47 increased both adipocytes differentiation with high lipid accumulation and high levels of reactive oxygen species (ROS). BDE 47 also induced mitochondrial ROS via significantly promoting mitochondrial respiration in adipocytes along with the improvement of glycolysis. Application of mitochondrial antioxidant or xanthine oxidase inhibitor suppressed BDE 47-induced ROS generation and lipid accumulation. Metabolomics profiling demonstrated BDE 47 induced similar metabolic profile as the positive control (Rosiglitazone). BDE 47 significantly altered purine metabolism and glutathione metabolism which was related to the increase of oxidative stress. BDE 47 also activated the conversion from xanthine to uric acid via xanthine oxidase to elevate the ROS production during adipocyte differentiation. In conclusion, the environmental pollutant, BDE 47, is capable of enhancing 3T3-L1 preadipocytes differentiation via altering the purine metabolism to induce oxidative stress.

P-196	Metabolomic signature in the plasma of patients with OPA1-related dominant optic atrophy reveals alteration of purine metabolism

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Dominant optic atrophy (DOA, MIM#165500), affecting the retinal ganglion cells and their axons forming the optic nerve, is associated in most cases with Optic Atrophy 1 gene (OPA1) pathogenic variants. OPA1 is a dynamin GTPase involved in mitochondrial inner membrane processing, with a defect of mitochondrial fusion and cristae organization as a consequence of mutations. A non-targeted metabolomics approach was applied to determine whether metabolic signature exists in DOA patients' plasma. Twenty-five patients with several pathogenic variants and twenty controls plasma samples were analyzed by ultra-high-pressure liquid chromatography coupled with high resolution mass spectrometry (UHPLC-HRMS), according to our validated method that allows the non-targeted exploration of 500 polar metabolites. Using this method, our results revealed a robust and significant metabolic signature in patients' plasma compared to controls. This study highlights a major impairment of the purine metabolism that may be related either to mitochondrial dysfunction or to the modified GTPase activity of OPA1. In addition, the altered concentrations of metabolites, deriving from the glycerol-phospholipids pathway to name one, were evidenced in patients. In conclusion, our metabolomics analysis, by screening a large set of molecules, provides for the first time a human OPA1 signature that not only contributes to further understand the pathology with new relevant features, but also offers new directions for therapeutic approaches. Overall this study shows that metabolomics is a powerful approach to explore mitochondrial and ocular diseases in plasma.

P-198	Environmental cadmium exposure induces urinary metabolite profile alterations in general Chinese pregnant women

PRESENTING AUTHOR: *Han Li, Huazhong University of Science & Technology, China*

CO-AUTHORS: *Yang Peng*

Cadmium, as a typical heavy metal, has become a major environmental threat to human health. It is already known that high-level cadmium exposure has adverse effects on human health. However, understanding regarding the health effects of low-level cadmium exposure on pregnant women remains unclear. The aim of this study is to investigate the urinary metabolic changes of pregnant women exposed to low-dose cadmium, and to identify effective biomarkers. Urine samples of 246 pregnant women were collected in the first trimester of pregnancy and were divided into three groups based on the tertile distribution of urinary cadmium levels which were determined using inductively coupled plasma mass spectrometry (ICP-MS). The alterations in the metabolite profile were measured by ultra-performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-QToF-MS). Cadmium-related metabolic biomarkers were investigated by comparing the samples of the first and third tertiles of cadmium exposure classifications using a partial least-squares discriminant model (PLS-DA). Eight potential biomarkers were identified, including L-cystine, L-tyrosine, dityrosine, cysteineglutathione disulfide, 16DHEA-S, prolylhydroxyproline, histamine and uric acid. The results showed that environmental cadmium exposure, even at low levels, could cause metabolite alterations in pregnant women which might be associated with adverse health outcomes. The findings may be valuable for the cadmium risk assessment for pregnant women.

P-199	MS-Probes and Stable Isotope Coding for Targeted Analysis of Short Chain Fatty Acids in Biological Fluids

PRESENTING AUTHOR: *Janine Cooney, Plant & Food Research Ltd, New Zealand*

CO-AUTHORS: *Tania Trower*

Growing evidence suggests a link between enteric bacteria and the central and enteric nervous systems - the gut-brain axis. Dysbiosis of the gut microflora influences these interactions and plays an important role in the pathophysiology of functional gastrointestinal and nervous system disorders1. Modulation of the balance of gut microbes through dietary intervention, particularly the use of probiotics and fibre, can affect production of short chain fatty acids (SCFAs), the end-products of microbial fermentation. SCFAs positively influence host metabolism by promoting energy homeostasis, regulating immune responses and epithelial cell growth, and promoting the functioning of the central and peripheral nervous systems2. To facilitate understanding of the complex interplay between diet, host-gut microbiota and human health, quantitative analysis of SCFAs in faecal samples is often used to monitor the efficacy of gut-health interventions. Because of the volatility of SCFAs, LCMS analysis is preferred over GCMS; however, accurate quantitation is hampered by the lack of commercially available internal standards (IS) to compensate for matrix effects prevalent in biological samples. Han and colleagues recently reported an MS-probe and stable isotope coding method for the accurate quantitation of SCFAs in human faeces3. This method uses 12C/13C6-3-nitrophenylhydrazine to convert SCFAs quantitatively to their 3-nitrophenylhydrazones, to increase their analysis sensitivity. Isotope label coding is enabled using 13C6-3NPH to create an IS for each SCFA. We have extended this methodology for analysis of SCFAs in other types of biological samples, including digesta and plasma, and will demonstrate applications in human studies and animal trials.

P-200	Explaining the Rye Factor by Metabolomics and Fluxomics

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Rye bread has been shown to produce a lower insulin response than wheat bread, even though their produced glucose responses are the same. This effect is called the "Rye Factor" and efforts have been made to understand its underlying mechanism. Our group has previously performed plasma NMR and LC-MS metabolomics in several bread intervention studies in order to illuminate the nature of the rye factor. Based on the previous metabolomics studies, we formed a hypothesis for the mechanism of the rye factor based on the differences in the rate of absorption of available carbohydrate. The hypothesis was tested using stable isotope flux analysis. Ten males were recruited for a crossover study in which rye/wheat breads were eaten under infusion of isotopically labeled glucose and blood samples were analyzed by targeted LC-MS metabolomics, from which the flux of glucose from the intestine to the blood was calculated. The rate of exogenous glucose appearance was found to be significantly higher after ingestion of refined wheat bread compared to wholemeal rye bread during the initial 15 minutes, but the plasma glucose concentrations did not differ between the different breads at any postprandial time point. Additionally, the total postprandial transport of glucose, expressed as the area under the curve (AUC) for rate of glucose appearance, was significantly higher after ingestion of wheat bread. The higher flux of glucose triggers a higher production of insulin in order to prevent hyperglycemia. These findings can elucidate the nature of the rye factor.

P-201	Automatic nuclear magnetic resonance spectroscopy (NMR) urinary analysis: a new approach for selective screening of inborn errors of metabolism (IEM)

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Background Selective screening of IEM is mainly performed by GC/MS, HPLC/MS, IEC and other methods. Work up of samples and methods necessary to make a diagnosis are time consuming and not all methods are applied in a sample with unspecific symptoms like "autism" or mental disability. After technology and automation in NMR analysis have greatly improved, we applied NMR as first line method in selective screening of IEM using ERNDIM proficiency testing for proof of principle. Methods To 900ul urine 10% buffer was added and analyzed with a Bruker IVDr System at 600 MHz. Spontaneous urine samples of 420 healthy children were used as reference. 16 urine samples of the ERNDIM proficiency testing program were analysed using a panel of 150 metabolites tested in the reference group. Results In 15 out of 16 the correct diagnose could be made:2 normal findings,6 Organo-,3 Aminoacidopathias,a MCAD- and Dihydropyriminidase-deficiency,a Sialidosis I and an Odontohypophosphatasia.A Glut I low excretor could also correctly be diagnosed. A patient with aromatic acid decarboxylase deficiency could not be diagnosed (as it was the case in 17 out of 20 laboratories. This became an "education sample").

P-202	Novel peroxidation products of adrenic, arachidonic, and docosahexaenoic acids in asphyxiated newborn's plasma

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During hypoxic ischemic insult, oxidative stress appears to play a crucial role in the biochemical processes that trigger neuronal damage. Lipid peroxidation products are the most widely accepted compounds as indicative of lipid damage by oxidative stress. In this work we present a validated liquid chromatography mass spectrometry determination of 30 isoprostanoids in newborn plasma samples covering a broad range of those oxygenated metabolites of Polyunsaturated Fatty Acids (PUFAs). The method was developed taking into account the specific requirements for its use in neonatology (i.e. limited sample volumes, straightforward sample processing, and high analytical throughput). The method was validated following stringent FDA guidelines and was applied to the analysis of 150 plasma samples collected from neonates with ischemic hypoxic encephalopathy. Data obtained from the quantitative analysis of isoprostanoids was critically compared to that provided by a previously developed approach aiming at the semi-quantitative detection of total parameters of oxygenated metabolites derived lipid peroxidation biomarkers.

P-203	Metabolic profiling reveals colonization-induced host-gut microbial interaction

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The colonization by microbiota (an ensemble of all symbiotic or pathogenic microorganisms that share human body space) has a major impact on the human host as it has been associated with numerous health outcomes. However, mechanisms underlying the interaction of microbe-derived metabolites with host signaling and metabolic pathways remain to be elucidated. As microbiota seems to have several important influences on physiological processes in human, particularly on energy metabolism and immunomodulation in the host, significant changes can be expected in metabolic profile with respect to diverse microbial colonization. Mass spectrometry-based metabolic profiling by selected reaction monitoring (SRM) assays was performed in biofluids to detect markers of microbial colonization, immune homeostasis and energy metabolism status. We have built SRM libraries to investigate primarily the microbial influence on tryptophan metabolism in urine samples (n=54) and amniotic fluid (n=58). As the result we have discovered previously unreported microbiota?associated metabolites, which are suitable markers of colonization and contribute novel insight into the host-microbiota metabolism. In parallel, we have developed methods to assess circulating endogenous markers of energy metabolism (i.e. TCA cycle, pyruvate, propionate, butyrate or fatty acid metabolism) and immune response to investigate either beneficial or harmful effects of microbial colonization on the host. This study is pioneering the transition from a descriptive to a mechanistic understanding of the human microbiota, which is critical not just for fully appreciating the impact of these organisms on host health, but also for harnessing this knowledge to treat disease. This work was supported by the GACR (#17-24592Y).

P-204	Evaluation of metabolic changes in lungs of tuberculosis-infected mice using new high resolution GC/Q-TOF

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CO-AUTHORS: *Mª Fernanda Rey-Stolle, Vineel Reddy, Adrie Steyn, Nathan Eno, Coral Barbas*

Tuberculosis (TB) is one of the major global health problems, with an estimated death of 1.5 million individuals annually. Using metabolomics, new TB biomarkers can be identified to make progress in our understanding of the disease as well as to improve the diagnostics. In this study, we used a mouse model of Mtb infection to determine the metabolic profile of uninfected and infected lung tissues. Mice were infected with 5x104 CFU of Mycobacterium tuberculosis (Mtb) H37Rv via the intratracheal route. The dried extracts were derivatized by O-methoximation followed by trimethylsilylation. GC/MS analysis was performed using an Agilent 7890B GC system coupled to a novel high resolution 7250 GC/Q-TOF equipped with EI source allowing low-energy ionization. The individual analytical fingerprints were obtained using the SureMass feature detection algorithm of Agilent MassHunter Unknowns Analysis. Statistical analysis was performed in Mass Profiler Professional (MPP). To identify new pathophysiological pathways involved in infection as well as biomarkers of TB, the untargeted metabolomics study was performed using uninfected and infected lung at 9 weeks following infection. After initial compound annotation, low-energy EI data were used to confirm the molecular ions and identify molecular formulas of putatively identified compounds and unknowns, respectively. Using the Fold Change analysis in MPP, alteration in amino acids and nucleobases profiles have been observed. In addition, a change in the profile of itaconic acid, a compound that likely plays a role in macrophage-based immune response, has also been detected.

P-205	Analytical performance of high-resolution accurate mass (HR-AM) quantification: A scale-up study of polar metabolites quantifiacation in glioblastoma cell cultures

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Liquid chromatography-high resolution mass spectrometry enables comprehensive and simultaneous qualitative / quantitative measurement of metabolites using accurate-mass (HR-AM) extracted ion chromatograms. However, variations in outcomes as the analytical process is scaled up have not been adequately reported. Herein, we evaluated the analytical performance of LC-HRMS for a representative metabolomic consumption and release (CORE) investigation that involved 988 analytical runs spread over 15-days. The test samples comprised of methanolic extracts from two glioblastoma cell sub-types grown in vitro with drug treatment. Specific calibration ranges and sample dilution factors were designed subsequent to qualitative full-scan mode screening of 34 metabolites that majorly included amino acids, glycolysis and tricarboxylic acid cycle intermediates. R2 ~0.94-0.99 was reproducibly obtained with the calibration curves for 31 metabolites establishing linearity across the respective concentration ranges. Accuracy, recoveries and inter-day precision was established using standard and matrix-matched spiked quality control samples. Recoveries for these QCs were found within 80-120%. Inter-day precision was determined using 26 replicates QC injections at regular intervals throughout the course of study. Recovery values with ?25% RSD for of 31 metabolites demonstrated reasonable inter-day precision and robustness of the method indicating it's fitness for the purpose. The outcome demonstrated, HR-AM quantification by LC-HRMS is an accurate and reliable approach that can be scaled-up for metabolomics studies involving significantly large sample runs over extended period of time. Additionally, using this method, differential CORE profiles indicating altered metabolism in two sub-types were identified and further highlighted using unsupervised principal component analysis (PCA).

P-206	Tissue metabolomic profiling reveals dysregulated metabolism in human esophageal cancer

PRESENTING AUTHOR: *Zhiyi Yang, Hong Kong Baptist University, Hong Kong*

CO-AUTHORS: *Zhongjian Chen, Xiaojiao Yi, Zhenyu Guo, Wenhao Ji, Yanjun Hong, Zongwei Cai*

Esophageal squamous-cell carcinoma (ESCC) is among the leading causes of cancer mortality worldwide. Previous research on human plasma metabolomics has revealed perturbations of several phospholipid species in ESCC patients. In this study, to further characterize the metabolomic signatures in ESCC, paired tumor and adjacent normal tissues from 85 ESCC patients were investigated by liquid chromatography—tandem mass spectrometry (LC-MS/MS)-based metabolomic profiling. Tumor tissues were clearly separated from the normal tissues based on the partial least-square discriminative analysis (PLS-DA) model (R2Y=0.880 and Q2Y=0.815 in positive mode). Over 40 biomarkers were identified to be significantly perturbed in tumor tissues. Major elevated metabolic pathways were beta-alanine metabolism, alanine, aspartate and glutamate metabolism, kynurenine metabolism and glycerophospholipid metabolism, which reveal the immunosuppressive nature of ESCC. Tissues from Stage III and Stage I patients also exhibited significant difference, which can provide evidence for cancer staging and diagnosis for ESCC. This study gives direct evidences of metabolism dysregulation in ESCC patients, and suggests the diagnostic potential of biomarkers in ESCC.

P-207	Protective effect of Centella asiatica extract (ECa233) in rotenone-induced liver injury rats as revealed by metabolomics analysis

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Liver diseases cause serious public health problems worldwide. Several studies of hepatoprotective activities from natural plant extracts have been reported during recent years. The aim of this study was to examine protective effect of the standard extract of Centella asiatica (ECa233) in rotenone-induced liver injury rats using a GC-MS based metabolomic approach. ECa233 contains >80% triterpenoids with a ratio of madecassoside to asiaticoside of 1.5(±0.5):1. Rats were randomly divided into three groups (with six rats/group): sham negative control, rotenone positive control, and the ECa233 test group. Rats in the ECa233 group received 10 mg/kg ECa233 orally for 20 days, followed by 2.5 mg/kg intraperitoneal rotenone injection to induce toxicity before being sacrificed. Metabolomic analysis showed that supplementation of ECa233 protected rat liver against rotenone toxicity. Pipecolinic acid was one of the most important metabolites; its level was decreased in the rotenone group as compared to control. Supplementation with ECa233 before administration of rotenone raised pipecolinic acid to levels intermediate between controls and rotenone alone. Antioxidant tests revealed that ECa233 inhibited lipid peroxidation and increased catalase activities in liver tissue. The outcomes of this study provide further novel evidence in support of using Centella asiatica as a value-added component in new dietary supplements or medicines for liver disorders in the future.

P-208	METABOLITES AND LIPIDS PROFILING AND IMAGING IN DIFFERENT TYPES OF HISTOLOGICAL PREPARATION OF THYROID CANCER TISSUE SECTIONS

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The distinction of different thyroid cancer types has important implication for clinical management. Classification based on histopathological features can be supported by molecular biomarkers, including metabolomic and lipidomic signatures, identified with the use of high-throughput mass spectrometry techniques. Formalin fixation and paraffin embedding is a standard procedure for preservation of tissue samples and constitute highly valuable source of clinical material for retrospective molecular studies. In this work we used GC/MS based molecular profiling of FFPE thyroid tissue for identification of metabolites characteristic for different thyroid cancer types. Moreover we performed imaging and profiling of lipids present in malignant and non-cancerous formalin fixed thyroid tissue. GC/MS-based molecular profiling from archival FFPE tissues was used to identify metabolites characteristic for different types of thyroid cancer. We revealed several metabolic signatures discriminating cancerous from normal tissue, as well as malignant thyroid cancers from benign follicular adenoma. Hence, the GC/MS-based metabolome profiling of FFPE thyroid specimens could be potentially used as an auxiliary diagnostic tool to support classification of thyroid cancers. High resolution MALDI-MSI technique was used for imaging of lipids in fresh frozen and formalin fixed thyroid tissue samples. Several phosphatidylcholine, sphingomyelin and phosphatidic acid species were detected as corresponding [M + H]+, [M + Na]+ and [M + K]+ ions. This results proved the viability of MALDI-MSI in search for lipid biomarkers directly in formalin fixed clinical material. This work was supported by the National Science Centre grants (No UMO-2013/08/S/NZ2/00868 and UMO-2012/07/B/NZ4/01450) and European Cooperation in Science and Technology (ECOST-STSM-BM1104-011014-046331).

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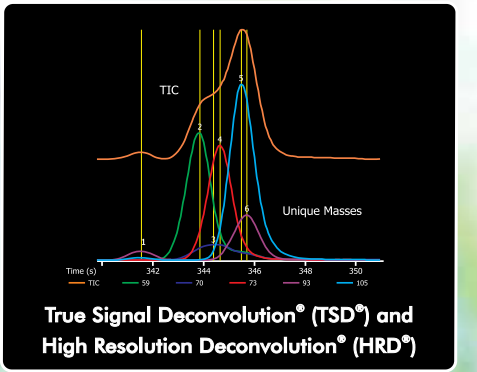

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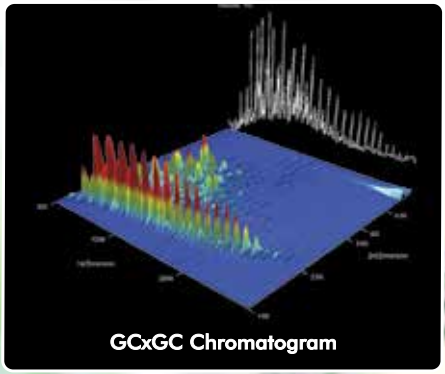

GCxGC Chromatogram

P-209	Plant sesquiterpene lactone derivative overcomes acquired vemurafenib resistance and reduces cutaneous side effect of vemurafenib in mice through modulating oxylipin dynamics and beyond

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Vemurafenib, a chemotherapeutic drug targeting BRAFV600E mutant melanoma, commonly causes cutaneous squamous cell carcinoma or keratoacanthomas side-effect in patients. We demonstrated that DETD-35 derived from a plant sesquiterpene lactone deoxyelephantopin can effectively reduce tumor growth in acquired vemurafenib resistance (A375-R) xenograft models, where vemurafenib showed no anti-tumor activity. We also observed that vemurafenib administration to DMBA/TPA-irritated mice (named DTV) promoted a much earlier onset of cutaneous papillomas formation in mice. Of note, DETD-35 treatment effectively reduced skin papillomas formation and numbers/sizes in DTV mice. Oxylipins are a group of lipid mediators playing critical roles in inflammation and cancerous diseases. LC-MS-based oxylipin metabolome study shows that vemurafenib affected the oxylipin profile and dynamics in DMBA/TPA-irritated mice. Of note, linoleic acid derived oxylipin metabolites, such as EpOMe(s) or DHOME(s) were elevated in DTV mice which can be significantly attenuated by DETD-35 treatment. The LOX/CYP450 enzymes responsible for those oxylipins production were found over-expressed in skin neutrophils/macrophages. Mechanistic study shows that DETD-35 inhibited the paradoxical activation of MAP kinases by vemurafenib in mouse skin or in HRAS mutant melanoma cells; furthermore, DETD-35 could also inhibit cell proliferation of HRAS mutant keratinocytes, which are observed abundantly in DMBA/TPA-irritated mouse skins. This study suggests that DETD-35 can be a novel adjuvant agent for vemurafenib in treatment of BRAFV600E mutant melanoma and reducing the drug side effect.

P-210	Biological and functional analysis of Coenzyme A biosynthetic pathway in enteric protozoan parasite Entamoeba histolytica

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CO-AUTHORS: *Ghulam Jeelani, Tomoyoshi Nozaki*

Entamoeba histolytica is an anaerobic parasitic protozoan causes amoebic dysentery. Metronidazole has been a drug of choice against amebiasis for decades despite its known side effects and low efficacy against asymptomatic cyst carriers. Therefore, identification and characterization of specific targets is needed to design new therapeutics for improved treatment against amebiasis. Coenzyme A (CoA) is an essential cofactor in many living organisms as an acyl group carrier and carbonyl activating group in numerous reactions central to cellular metabolism. The biosynthesis of CoA from pantothenic acid is an essential universal pathway in prokaryotes and eukaryotes. E. histolytica also rely on the uptake of exogenous pantothenate. Using comparative genomics, we have identified genes involved in biosynthesis of CoA from pantothenate in E. histolytica: Pantothenate kinase (EC 2.7.1.33), bifunctional phosphopantothenate-cysteine ligase/decarboxylase (EC 6.3.2.5/ EC 4.1.1.36), pantetheine phosphate adenylyltransferase (EC 2.7.7.3) and dephospho CoA kinase (EC.2.7.1.24). In order to further characterize the importance of this pathway, we have utilized the epigenetic gene silencing strategy. Using this strategy, we were able to repress pantothenate kinase (PanK), dephospho CoA kinase 1 (DPCK1) and dephospho CoA kinase 2 (DPCK2). Repression of individual above genes showed severe growth defect, significantly decreased in CoA concentration suggesting the essentiality of this pathway in this parasite. To better understand the role of unidentified metabolic system and/or change in metabolic capacity after silencing of PanK and DPCK gene, we performed metabolomic analysis of the above knock down strains. Significance of metabolomic changes after knocking down above CoA pathway genes will be discussed.

P-211	Effect of tomato interventions on the plasma lipid profile of men pre-prostatectomy

PRESENTING AUTHOR: *Morgan Cichon, The Ohio State University, United States*

CO-AUTHORS: *Ken Riedl, David Francis, Steven Clinton*

Diets rich in tomatoes have been associated with a decreased risk of chronic diseases, including prostate cancer. However, the mechanism behind this observed protective effect remains unclear. Given the lipid-like structure of lycopene, one of the bioactive components of tomatoes, we are interested in the effect of tomatoes on the human lipid profile, which has not yet been evaluated in detail. Here we investigated the effects of red and tangerine tomato juices on the plasma lipid profile as a way of better understanding a mechanism of action. In addition to the traditional red tomato, a unique tangerine tomato variety was selected due to its different phytochemical profile, in particular, differing carotenoid composition and content. In this study, men (n=45) with prostate cancer scheduled for a prostatectomy consumed red tomato juice, tangerine tomato juice, or a low lycopene diet for 3-4 weeks prior to surgery. Baseline and post-intervention plasma samples were analyzed using a differential ion mobility mass spectrometry-based lipidomics platform covering 13 classes of blood lipids (approximately 12,000 lipid species). The fully quantitative data was statistically analyzed to determine classes and species of lipids altered in response to the red and tangerine tomato juice interventions. Results suggest significant decreases in phospholipid and lysophospholipid classes with tomato intake. There also appear to be a number of specific triacylglycerides that decrease in plasma following the tomato interventions. We continue to mine the data to confirm lipid differences and determine if these effects vary based on tomato variety.

P-212	Understanding asthma-COPD overlap syndrome: Exhaled breath condensate analysis using (1H) NMR based metabolomics approach

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Asthma and chronic obstructive pulmonary disease (COPD) are two common obstructive lung diseases which have been extensively investigated. However, asthma-COPD overlap syndrome (ACOS), where patients exhibit features of both asthma and COPD, remains a weakly defined clinical entity. No specific biomarker exists to differentiate ACOS from asthma or COPD. Metabolomics of exhaled breath condensate (EBC) has emerged as a popular non-invasive tool to help understand underlying pathophysiologies of various respiratory diseases. This is the first study where we hypothesize that metabolomic analysis of EBC will help classify ACOS better and ascertain whether this overlap syndrome has its own unique pathophysiology. EBC samples were collected from patients with asthma (n=23), COPD (n=26), ACOS (n=31) and healthy controls (n=27). Proton NMR spectra of EBC samples were acquired using 800 MHz Bruker Avance III spectrometer equipped with a CryoProbe. Following pre-processing of the spectra, both multivariate and univariate analysis was applied. On comparing asthma, COPD, ACOS and controls, an optimum classification of ACOS was obtained with the OPLS-DA model. Finally, receiver operating characteristic curve could identify with highest accuracy the metabolites responsible for distinguishing ACOS. Metabolites including lactate, pyruvate, propionate, ethanol and fatty acid were found to be significantly dysregulated in ACOS patients. In conclusion, the distinct metabolic signatures of ACOS are strongly suggestive of the disease having a distinct clinical identity, as compared to asthma and COPD. These findings pave way for validation studies which can help unravel this complex overlap syndrome better.

P-213	Serum lipid profiling reveals the effect of dietary Chrysanthemum morifolium Ramat leaf extracts on high-fat diet induced obesity in a mice model

PRESENTING AUTHOR: *Jong Cheol Shon, Kyungpook National University, South Korea*

CO-AUTHORS: *Won Cheol Kim, Kwang-Hyeon Liu*

The Chrysanthemum morifolium Ramat has been known to have a various pharmacological effects such as anti-obesity and anti-diabetes. Lipidomic biomarkers responsible for the pharmacological effects on leaf of Chrysanthemum morifolium Ramat extracts (CME) were investigated in serum of mice fed high-fat diets (HFD). In brief, the serum of mice fed a normal diet (ND), a HFD, a HFD plus CME 1.5% diet (CLL), and a HFD plus luteolin 0.003% diet (LU) for 16 weeks were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS) combined with multivariate analysis. The ND, HFD, CLL, and LU groups were clearly discriminated upon partial least-squares discriminant analysis (PLS-DA) score plot, and the major metabolites contributing to such discrimination were triacylglycerols (TAGs), cholesteryl esters (CEs), phosphatidylcholines (PCs), ceramides (CERs), sphingomyelins (SMs), and lysophosphatidylcholines (LPCs). The levels of PCs, LPCs, SMs, and CERs were increased in HFD groups compared to ND groups and these lipids were recovered after administration of CME 1.5% or luteolin 0.003% extracts. The mRNA expression levels of ceramide synthase 6, sphingomyelin synthase 1, and choline phosphotransferase in CLL groups were also recovered to the levels of ND groups. In conclusion, these lipid-markers might be used to evaluate the anti-obesity effect of CME in mice. This study was supported by the Bio-Synergy Research Project (Grants NRF-2014M3A9C4066459) of the Ministry of Science, ICT & Future Planning through the National Research Foundation of Korea (NRF).

P-214	Metabolomic Analysis of Nicotinamide Phosphoribosyltransferase (NAMPT) Inhibition on PC3 Cancer Cell Line

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CO-AUTHORS:

Nicotinamide phosphoribosyltransferase (NAMPT) is the rate-limiting enzyme that catalyzes the first step in the biosynthesis of NAD from nicotinamide. Recent studies have demonstrated that NAMPT-mediated NAD biosynthesis in cancer cells plays a crucial role in several physiological processes. The down-regulation of NAMPT suppresses tumor cell growth in vitro and in vivo and sensitizes cells to oxidative stress and DNA-damaging agents. FK866, found to be bound to the nicotinamide binding pocket of NAMPT, specifically inhibits NAMPT in the cell and exhibits anti-tumor activity in preclinical tumor models. Thus, FK866 appears to be an ideal tool molecule for assessing the physiological function of NAMPT in the cell. GMX1778 is another important pillar in the elevation of NAMPT inhibitors to potential therapeutic agents. In the current study, further studies will be performed to assess the global effects of NAMPT inhibition by FK866, GMX1778 and their synergistic effects on cancer metabolism by using global mass spectrometry–based metabolic profiling. We demonstrated that metabolites associated with glycolysis, pentose phosphate pathways and TCA cycle are affected in the same fashion under the inhibition of FK866 and GMX1778. Betaine, phosphotidylcholine, diadyglycerol, phosphoethanolamine, phosphatidylinositol, phosphotidylglycerol, ceramide and sphingomyelin (SM) were observed to be up-regulated, which suggests there is cell-specific response of lipid metabolism to NAMPT inhibition with FK866 and GXM1778. NAMPT inhibition significantly modifies purine metabolism and pyrimidine metabolism under FK866 treatment, but not under GMX1778 treatment. The difference in metabolic changes under FK866 and GXM1778 might due to difference in structure and binding to NAMPT.

P-215	Integration of 1H NMR dereplication algorithm and MS/MS Molecular Networking for the identification of bioactive secondary metabolites

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CO-AUTHORS: *Denise Selegato, Alan Pilon, Rafael Freire*

Dereplication is of fundamental importance in current bioprospecting programs, emerging as a rapid way of identifying known compounds in mixtures, accelerating the selection and identification of biologically promising compounds. New methodologies have been developed, using multivariate analysis and pattern recognition algorithms, such as MS/MS Molecular Networking and Principal Component Analysis (PCA). The main goal of this work was to analyze the metabolic profiles of Fusarium solani and F. oxysporum isolated from Senna spectabilis's rhizosphere, aiming to dereplicate important and bioactive metabolites. We have integrated two different methodologies applied to 1H-NMR-based metabolomics and tandem mass spectrometry. By applying the first strategy, it was possible to identify and mathematically separate 1H-NMR peaks responsible for F. solani and F. oxysporum metabolic differentiation, identifying three known molecules fusaric acid, produced majorly by F. oxysporum, a mycotoxin with high commercial value, and two enniatins: HA23 and beauvericin, produced by F. solani and F. oxysporum, respectively. Complementarily, by target analyzing of all metabolites identified above using Molecular Networking, we were able to identify eight other analogues six enniatins, three of them known molecules and firstly described for F. oxysporum: beauvericin F, beauvericin D and beauvericin G2; and three firstly reported depsipeptides as well as three picolinic acid analogues.

P-216	Untargeted metabolomic analysis of porcine serum and oral fluid following intramuscular administration of a porcine reproductive and respiratory syndrome virus (PRRSV) modified-live virus (MLV) vaccine

PRESENTING AUTHOR: *Dr. David Borts, Iowa State University, United States*

CO-AUTHORS: *David Borts, Jeffery Zimmerman, Yuly Henao Diaz, Luis Giménez-Lirola, Ting-Yu Cheng*

First identified in 1991, porcine reproductive and respiratory syndrome virus (PRRSV) remains an economically important cause of reproductive failure in breeding stock and respiratory illness and death in young pigs. Understanding the pathogenesis of PRRSV and devising methods for controlling its effects remain active fields of study. In this experiment, an untargeted metabolomics approach was used to detect small molecule biomarkers associated with the replication of PRRSV MLV vaccine. Serum and oral fluid samples from 12 pigs collected prior to and following vaccination were analyzed. Samples were extracted by protein precipitation and analyzed using ultra-high-pressure liquid chromatography (UHPLC) coupled with high resolution orbitrap mass spectrometry. Multivariate statistical analyses revealed well-separated clusters between the pre-vaccination and post-vaccination groups in both serum and oral fluid specimens. Variable importance in projection (VIP) plots were used to rank metabolites for their ability to discriminate between these two groups. Receiver-operator characteristic (ROC) curves were calculated to determine the quality of biomarker sets. The resulting ROC curves suggested that the putative biomarkers identified in this study might form the basis of an effective diagnostic test for detecting PRRSV replication. Additional work will focus on metabolite identification, identification of small molecule biomarkers of PRRSV infection, and on potential diagnostic applications, e.g., discrimination between PRRSV vaccinated vs wild-type infected animals and detection of persistently infected animals.

P-218	Phenotyping analysis of a 5-compound herbal mixture on macrophage cell line using metabolomics analysis

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CO-AUTHORS: *Chika Shimobori, Akinori Nishi, Takashi Matsumoto, Nagisa Komokata, Hirotaka Kushida, Masahiro Yamamoto*

Combination drug like traditional herbal medicine (THM) is expected to overcome limitations of single agents through their synergistic or additive effects produced by various compounds. However, the modes of action (MoA) of THM remain unclear due to the complex nature of THM. To clarify the MoA of THM, it is essential to understand which component could be involved in the pharmacological action and what could happen in the body, tissues, and cells by administration of THM. In this study, we selected the bioavailable compounds from the components in maoto, which is a Japanese traditional medicine and widely used for the febrile symptoms induced by viral infection, and evaluated the influence of a selected 5-compound mixture (MIX5) on macrophage cell line using wide-targeted metabolomics analysis. We used RAW264.7 stimulated with poly I:C (PIC), a viral mimic, to evaluate the effects of MIX5 containing ephedrine, prunasin, cinnamic acid, glycyrrhetinic acid, and isoliquiritigenin, which could be detected in the systemic blood after oral administration of maoto in rat and human study. MIX5 suppressed interleukins and prostaglandin D2 production induced by PIC. Metabolomics analysis revealed that PIC induced metabolic reprogramming following activation of macrophage. MIX5 increased anti-inflammatory mediators such as omega-3 fatty acid metabolites without affecting metabolic reprogramming induced by PIC. Additionally, the effect of MIX5 on the lipid mediators was quite different from aspirin, one of the representative cyclooxygenase inhibitors. These results suggest that MIX5 exerts unique anti-inflammatory activity, the MoA of which is different from those of conventional anti-inflammatory drugs like NSAIDs.

P-219	Salmonella typhimurium Infection Reduces Schistosoma japonicum Worm Burden in Mice

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CO-AUTHORS: *Xiaoyang Zhu, Huiru Tang*

Coinfection of microorganisms is a common phenomenon in humans and animals. In order to further our understanding of the progress of coinfection and the possible interaction between different pathogens, we have built a coinfection mouse model with Schistosoma japonicum and Salmonella typhimurium, and used this model to investigate the systemic metabolic and immune responses using NMR-based metabonomics and immunological techniques. Our results show that Salmonella typhimurium (ATCC14028) infection reduces adult schistosomal worms and eggs, relieves symptoms of schistosomiasis and also abates the mortality of mice infected by Schistosoma japonicum. In addition, Salmonella typhimurium infection counteracts the metabolic disturbances associated with schistosomiasis. Furthermore, immune analyses also indicate that shift of the immune response to different pathogens is a result of indirect interactions between Schistosoma japonicum and Salmonella typhimurium within the host. Salmonella typhimurium infection can ameliorate Schistosoma japonicum-caused schistosomiasis in BALB/c mice, which is most likely due to inverse immune polarization. Our work provides an insight into coinfection between Schistosoma japonicum and Salmonella typhimurium, and may further contribute to the development of new tools for controlling Schistosoma japonicum-associated diseases.

P-220	Metabolomic analysis of Haemonchus contortus infection in sheep

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CO-AUTHORS: *Horst Schirra, Gene Wijffels*

The helminth Haemonchus contortus or "The Barber's Pole" worm has been previously shown to be highly pathogenic to small ruminants such as sheep and goats causing anaemia, oedema, diarrhoea and even death if left untreated. This parasite, which uses sheep as a host system to feed and multiply, causes substantial economic losses worldwide. Furthermore, due to the exclusive and overuse of drug therapy (anthelmintics) H. contortus has become highly and multi-drug resistant. Therefore, new and sustainable control strategies have become highly sought after. In this project we investigated the changes in metabolite concentrations in two lines of sheep selected for resistance or susceptibility during the H. contortus host-infection stage. The data was initially analysed univariately before 2D and 3D multivariate clustering and trajectory plotting was undertaken. Metabolites of similar trajectories were grouped by means of Effect Projections (EP) and Hierarchal Cluster Analysis (HCA). Individual metabolites were analysed based on the data from the 1-, 2- and 3D trajectory plots. These analyses provide insights into the metabolic responses in the resistant and susceptible hosts during the course of a H. contortus infection.

P-221	COnsortium for METabolomics Studies (COMETS): Developing Resources to Accelerate Scientific Discovery

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Metabolomics is an emerging approach used for epidemiologic and clinical studies, which has the potential to improve disease risk assessment, screening, diagnosis, prognosis and predictive response to therapy, as well as provide disease mechanistic insight. In recent years, there have been an increasing number of epidemiologic studies that use metabolomics to explore metabolite associations with a wide array of disease-related traits and outcomes. It is timely to establish mechanisms for leveraging existing resources and data for novel biomarker discovery using metabolomics approaches. To this end, the National Institutes of Health COnsortium of METabolomics Studies (COMETS) was established in 2014 (http://epi.grants.cancer.gov/comets/), and currently includes 39 prospective cohorts and 2 consortia from the United States, Europe, Asia and South America. The COMETS mission is to promote collaborations among prospective cohort studies that have measured metabolites and followed participants for a range of health outcomes. COMETS aims to facilitate an open exchange of ideas, knowledge, and results to accelerate a shared goal of identifying metabolomic profiles associated with disease phenotypes (e.g. heart disease, diabetes, and cancer). Here we describe the COMETS structure and progress of the consortium to advance the use and impact of metabolite profiling in population-based research. Briefly, we will highlight efforts in gathering metabolomics data from different studies and analytical platforms to enable pooled analyses. This includes the development of COMETS Analytics, which is a secure, user-friendly, web-based analytical platform that promotes reproducibility and data autonomy by harmonization of metabolite identifiers across consortiums and methods.

P-222	Programmed cell death driven by up-regulation of phospholipids in cancer cells: A potential anticancer compound from Bornean Dysidea sp.

PRESENTING AUTHOR: *Yoong Soon Yong, Biotechnology Research Institute, Universiti Malaysia Sabah, Malaysia*

CO-AUTHORS: *Ping Chin Lee, Hsin-Chang Chen, Yee Soon Ling*

Marine sponges' rich in pharmacologically-active chemicals, have been demonstrated to exhibit anticancer properties for decades. Classical natural product chemistry is evolving to modern-day metabolomics as a result of the advent analytical platforms and sensitive instrumentation. Vast number of compounds had been discovered from these organisms to overcome drug resistance while reducing side effects. High-throughput metabolomics enables researchers to identify the metabolites where overall comparison between the treated subjects and control could be done. Throughout such analysis, the underlying dysregulated metabolites pathway could be mapped to understand the overall effects. Current study, we exposed cell line SK-Hep1 (human hepatic adenocarcinoma) with compound isolated from Bornean Dysidea sp.. High-throughput lipidomics revealed that the cell underwent programmed cell death after treated with 6.5 µg/mL of isolated compound. XCMS revealed a total of 58-lipids were significantly (p-value <0.05) perturbed. Among them are diacylglycerols and phospholipids, including phosphtatidylethanolamines (PE), phosphtatidylserines (PS), phosphtatidic acids (PA), and phosphtatidylinositols (PI). Upregulation of these lipids were reported to have strong correlation with programmed cell death (apoptosis). Cyto-histologic observation using propidium iodide and hoechst co-staining method suggested the cells underwent apoptosis. Additionally, it is interesting that ubiquinone derivatives, including 3-demethylubiquinol-10 (reduced form of Coenzyme Q10) and 6-methoxy-3-methyl-2-all-trans-decaprenyl-1,4-benzoquinol were upregulated suggesting that the cell mitochondrial undergoing substantial oxidative stress which eventually leading to cell death. However, the exact cell death mechanism is pending to be illustrated. To conclude, we discovered a promising anticancer compound from Bornean Dysidea sp.. Furthermore, in vivo study is necessary to evaluate the cytotoxicity of the compound.

P-223	Elevated plasma metabolites reveal actively regulated sphingomyelin-ceramide metabolic pathway in obesity

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CO-AUTHORS: *Eric Tzyy Jiann Chong, Yee Soon Ling*

Obesity is associated with increased risk of several chronic diseases, including stroke, heart attack, type 2 diabetes and cancer. Globally, more than 600 million individuals are obese. This study aims to investigate the plasma metabolite differences between obese and lean individuals. Non-polar plasma metabolites were extracted from 50 obese (BMI ? 30) and 50 lean (BMI < 25) individuals using liquid-liquid extraction method, and profiled using liquid chromatography followed by Orbitrap mass spectrometry technologies. The untargeted metabolite datasets were analyzed through multivariate approaches and matched to the LIPID MAPS® Database. Our study revealed that 11 lipid species were significantly elevated in obese than in the lean individuals, and most of these lipid species are major components in the sphingomyelin-ceramide metabolic pathway. This study suggests that the sphingomyelin-ceramide metabolic pathway is actively regulated in obese individuals; the finding is useful in pathway inhibitor discoveries for future obesity prevention and management.

P-224	Development of biomarkers for the protein restriction using Slc6a19 knock out mice

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CO-AUTHORS:

Mutation in BoAT1 (Slc6a19) lead to an autosomal recessive disorder known as Hartnup disorder that is mainly characterized by reduced absorption of amino acids in the intestine and renal aminoaciduria. The characterization of Slc6a19 knock out mouse model has revealed signs of protein restriction such as elevated levels of FGF21, but also higher levels of GLP-1 due to increase in amino acid load in the intestine. The combination of both effects improve glycaemic control and makes this transporter a potential target for the treatment of type 2 diabetes. Our lab is currently screening for chemical compounds that can block this transporter effectively. To evaluate the efficacy of these compounds, there is a need to develop biomarkers that can easily detect the successful inhibition of BoAT1 in mice and to detect protein restriction in general. To find biomarkers that can detect the inhibition of BoAT1 in intestine and correlate with protein restriction and malabsorption, a non-targeted metabolomics approach was designed to identify metabolites in urine, fecal and breath samples. A particularly interesting group of metabolites are fermentation products of amino acids that may show proportionality to protein load. The microflora of the intestine ferments amino acids into short chain fatty acids and other metabolites. In urine samples, a significant increase in neutral amino acids and some metabolites of bacterial origin was seen in the knock out mice. This shows the potential of qualitative metabolomics to identify biomarkers for protein restriction and malabsorption.

P-225	Wine, Health and Metabolomics: Consumption of dealcoholised wine with resveratrol, changes the human plasma profile

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Resveratrol has been linked to positive impacts on human health following its consumption and so it is one of the most highly studied bioactive phenolic compounds. Of specific interest is its presence in wine which is a main dietary source of resveratrol and its contribution to the potential benefits of moderate wine consumption. Research has demonstrated that the bioavailability of resveratrol, in humans, is relatively low.1 Some studies have reported the formation of known resveratrol conjugates such as resveratrol mono glucuronides and sulfates, but not all biochemical changes that resveratrol undergoes during metabolism are clear. For this study, metabolite changes that occur in human plasma following the consumption of resveratrol in dealcoholized wine were monitored using an untargeted metabolomics approach using reverse phase HPLC-HRMS (ESI negative mode). Bioinformatics tools including XCMS and CAMERA were used for the extraction of over a thousand molecular features. The data was explored using multivariate statistical methods and identification of metabolites was carried out using in-house spectral libraries and in-house reference standards. For characterisation of unknown metabolites, additional MS/MS experiments were performed for further structural information. The focus of the presentation will be on the metabolomics tools used to monitor resveratrol derived metabolites. In addition, the observed compositional changes that have been observed in human plasma as a result of the consumption of resveratrol will be outlined. Walle, T. et al. (2004) Drug Metabolism and Disposition, 32, 1377–82.

P-226	Salivary metabolomics for various cancer detection

PRESENTING AUTHOR: *Masahiro Sugimoto, Tokyo Medical University, Japan*

CO-AUTHORS:

Salivary tests facilitate frequent use since its non-invasive sample collection, which would enhance the opportunity of various diseases at early stage. Salivary metabolomics have shown its potential to detect various cancers, including oral, breast, and pancreatic cancers. However, sample collections should be carefully designed to reduce unexpected bias, and rigorous validations is necessary to manifest the biological link between the cancer-specific metabolic aberrance in tumor and salivary metabolite markers. Here, we identified metabolomic salivary markers for oral cancer detections. We utilized capillary electrophoresis time-of-flight mass spectrometry to quantify hundreds of metabolites in tumor and matched healthy tissues obtained from the patients with oral cancers. We also collected saliva samples from these patients and healthy controls. Totally, 17 metabolites showed consistently elevated in oral cancer saliva and tumor tissues in oral cancer patients. Of these, the combination of two metabolites showed high discrimination ability of oral cancer from healthy controls using saliva. We also collected salivary samples three times from oral cancer patients with different duration between saliva collection and the last diets. We evaluated which metabolites are sensitive or robust against the timing of sample collection. The identified markers showed a clinical feasible as non-invasive oral cancer screening.

P-227	An untargeted metabolomics approach to identify new pathways involved in Aspirin effects

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CO-AUTHORS: *Chiara Manega, Benedetta Porro, Alessandro Di Minno, Sonia Eligini, Donatella Caruso, Elena Tremoli, Viviana Cavalca*

Aspirin is one of the most commonly antiplatelet drug employed for cardiovascular disease. Although its clinical efficacy has been well documented, a substantial inter-individual variability in drug response exists, resulting in some cases in persistent platelet reactivity and even in atherothrombotic events. The mechanisms at the basis of this relevant variability remain poorly understood. Pharmacometabolomics, allowing to investigate the changes in the metabolome induced by Aspirin, can aid to establish the modified pathways and to elucidate the determinants of drug responsiveness. In this study we defined an urinary metabolic profile of healthy subjects (n=7) before and after 7 days of low–dose Aspirin treatment, through a liquid chromatography – time of flight mass spectrometry platform, in positive and negative ionization mode. Many metabolites showed a statistical significant difference (p<0.05) after Aspirin assumption (35 in positive ionization mode and 44 in negative ionization mode). Pathway analysis, performed on identified metabolites, reveals how histidine and purine metabolisms are greatly affected by drug exposure; in particular, several metabolites showed lower levels after pharmacological treatment. Interestingly, some of the metabolites of these pathways are involved in platelet aggregation and systemic inflammatory response. Furthermore, we observed the decrease of four different short-chain acylcarnitines, suggesting an increase in ?-oxidation mitochondrial process. The results presented in this report reveal relevant pathways altered by Aspirin treatment, which may provide important information to understand the multiple effects that contribute to drug response.

P-228	NMR-based Metabolomic Profiling of Blood Plasma at The Taiwan Biobank: Comparison between Normoglycemic And Prediabetic Subjects

PRESENTING AUTHOR: *Chung-ke Chang, Taiwan Biobank, Institute of Biomedical Sciences, Academia Sinica, Taiwan*

CO-AUTHORS: *Chen-Yang Shen*

The Taiwan Biobank (TWB) operates the recruitment, monitoring and data generation of a general population cohort with no history of cancer and is one of the premier scientific infrastructure projects in Taiwan. We compared the metabolic profile of carefully selected blood plasma specimens from normoglycemic and prediabetic participants in the TWB using NMR-based quantitative profiling. By linking the metabolomic data with information obtained from biochemical tests and questionnaires, we observed two interesting trends: (1) In some populations, ?-hydroxybutyrate levels were correlated to exercise status, which in turn was associated with plasma glucose levels. (2) Subjects with relatively high blood plasma ethanol/methanol levels were less likely to be prediabetic. The first trend confirmed a number of findings reported in the literature, whilst the second one was unexpected and may provide clues into the mechanism of the prediabetic condition. We also tested a number of random samples at different magnetic fields, and found that quantitative profiling of filtered blood plasma was generally reproducible, raising the possibility of using cheaper lower-field spectrometers for multi-site replication and data comparison. Our results suggest that biobanking of NMR-based metabolomic spectra coupled with quantitative profiling may provide a robust and reproducible database for research and data dissemination.

P-229	Metabolomics Analysis of Tuberculosis Drug Activity Using Q-TOF LC/MS

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CO-AUTHORS: *Travis Hartman, Steven Fischer, Kyu Rhee*

Pyrazinamide (PZA) is a frontline drug used in the treatment of tuberculosis. Unfortunately, widespread use has resulted in growing rates of PZA resistance, while knowledge of PZA's mechanism of action remains unresolved. Existing knowledge indicates that PZA is a prodrug that undergoes conversion to pyrazinoic acid (POA), the pyrazine analog of nicotinic acid, by the NAD salvage pathway-specific enzyme nicotinamidase (PncA). Knowledge of the downstream biochemical consequences of POA production however remains incomplete. NAD biosynthesis and NAD-dependent reactions are two potentially direct, but understudied, biochemical targets of PZA. The reasons for this are both biological and experimental in nature but due, in part, to limitations of existing analytical methodologies that have hindered broad and sensitive detection of cellular metabolites. Here we present a high performance Q-TOF LC/MS method that enabled the biologically unbiased study of the impact of PZA on the Mycobacterium tuberculosis metabolome. This method with its excellent retention time reproducibility, compound separation and Q-TOF mass accuracy was outstanding for metabolomics profiling experiments. Batch feature extraction and multivariate statistical analysis was used to discover activity-specific metabolic changes that may help explain PZA's unique therapeutic activity.

P-230	Development of simultaneous screening method of ten cytochrome P450 enzyme activity in human liver microsomes using P450 substrate cocktails and LC-MS/MS

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The early detection of potential drug interactions is an important issue of drug discovery that has led to the development of screening methods for potential drug interactions. In this study, we developed a validated screening method for potential drug interactions of inhibitory drugs for ten human P450 enzymes (CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 2E1, 2J2 and 3A) using two P450-isoform probe substrate cocktails and tandem mass spectrometry. The two P450 substrate cocktails were developed to minimized mutual drug interactions among P450 probe substrates. In the incubation study of these cocktails, the reaction mixtures were pooled and simultaneously analyzed using LC-MS/MS. The method was validated by comparing the responses for the metabolites in incubations of each single substrate to those in incubations with the two substrate cocktails. The change in each P450 enzyme activity was less than 20% in each cocktail set compared to the individual incubation. The method was also validated by comparing the inhibition data obtained from the incubation of each individual probe substrate alone with data from the cockail method. The IC50 value of each inhibitor in the cocktail agreed well with that of the individual probe drugs. As a screening method for potential interactions of the inhibition of these ten P450 isoforms, this method will be useful in the drug discovery process and for the mechanistic understanding of drug interactions.

P-231	Comprehensive quantitative metabolomic profiling to investigate metabolic alterations in invasive ductal carcinoma of the breast

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Invasive ductal carcinoma (IDC) is one of the most common type of breast cancer with the prevalence of approximately 80%. Identifying metabolic alterations associated with IDC will be helpful in understanding molecular events involved in IDC progression. This study aims to identify IDC specific metabolic alterations and discriminating IDC subjects from benign and controls based on these metabolic alterations. Metabolic profiles of serum (75 IDC, 33 benign and 33 healthy controls) and tissue (25 IDC, 25 benign and 25 normal) samples were acquired using targeted liquid chromatography-multiple reaction monitoring/mass spectrometry (LC-MRM/MS) and untargeted gas chromatography-mass spectrometry (GC-MS). Univariate and multivariate statistical analysis were performed in order to identify statistically significant metabolites involved in IDC. Comparative analysis of IDC against control subjects revealed significant (p-value<0.05) alteration of 54 metabolites in serum and 65 metabolites in tissue. It is notable that some metabolic alterations in serum including amino acids, nucleotides are correlated with tissue. Further, comparison between three groups depicted that some of the metabolic alterations are distinctive to IDC. Interestingly, some metabolic alterations in IDC were comparable to benign subjects and revealed a pattern of increment or decrement from benign to IDC samples suggesting their possible role in benign to malignant transformation. Pathway analysis of significant metabolites associated with IDC samples majorly showed involvement in pyrimidine metabolism, alanine, aspartate and glutamate metabolism, purine metabolism, D-glutamine and D-glutamate metabolism, fatty acid biosynthesis, glutathione metabolism. This study identified IDC associated metabolites which can be helpful in understanding IDC disease progression and therapeutic solutions.

P-232	Plasma metabolomics in postmenopausal Chinese-Singaporean women with low and normal bone mineral density

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The application of metabolomics may lead to the discovery of novel biomarkers with the potential to predict bone loss. In the present study, we applied untargeted metabolomics in combination with multivariate analysis to identify molecular changes in the blood of postmenopausal Singaporean women with low and normal bone mineral density (BMD). Plasma samples from 97 women were analysed with positive ionisation mode by using hydrophilic interaction liquid chromatography mass spectrometry (HILIC-MS). Serum C-telopeptide of Type I collagen (CTx-1) was measured by using a sensitive and specific enzyme linked immunosorbent assay (ELISA). In addition, dual energy X-ray absorptiometry (DXA, Discovery A, Hologic, WI, USA) was used to measure bone mineral density (BMD) of the femoral neck. Overall 127 metabolites were detected via HILIC-MS of plasma samples; however, multivariate analysis were not able to distinguish differences between BMD and the metabolome of postmenopausal Singaporean women. Hypoxanthine and threonine levels were significantly different between low and normal BMD groups (P < 0.05). Alanine, arginine, cysteine, creatine, creatinine, histidine, homoserine, kynurenine, glutamine lysine, phenylalanine, proline, tryptophan and 4-aminophenol levels were lower in the low BMD group when compared with the normal BMD; however; these differences did not reach statistical significance. Stepwise linear regression identified 2 and 5 metabolites associated with BMD (R2=8.58%) and CTx-1 (R2=15%), respectively. Pathways analysis revealed that these significant metabolites are associated with amino acid pathways such as threonine, glutamine, arginine and proline metabolism. Taken together, these findings suggest tentative evidence that there are changes in amino acid metabolism in osteoporosis.

P-233	Metabolic and transcriptomic profiling to investigate clonal evolution of metabolism in MCF7 cells

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Shifts in cancer cell metabolism coincide with changes in cell morphology, proliferation, and tumor metastasis. These changes are not only driven by genetic mutations, but also by adaptation to the microenvironment of tumor cells, including the availability of nutrients. A cell's ability to adapt its metabolism to changing conditions can drive the clonal evolution of the tumor, potentially resulting in more invasive or aggressive cells. This clonal evolution is often observed in the lab, where long term sub-culturing leads to development of a phenotypically distinct cell population. Therefore, it is important to understand how different cancer cells respond to different environments, both for the integrity of in vitro studies and for more precise prognosis and treatment. To investigate this dynamic process, we compared the transcriptomes and metabolomes of a morphologically distinct variant of the MCF7 adenocarcinoma cell line, which had been long cultivated in a nutrient-rich medium formulation, against commercially available "stock" cells. We then switched the media of each cell line to see if we could induce the other's transcriptional and metabolic profiles over successive passages using RNA-seq and proton NMR metabolomics. We predict that we will observe some plasticity in the stock cells' metabolism, but that the divergent cells have been driven to a "point of no return," where they become unable to adapt to limited nutrients. This has potentially important implications not only for the consideration of cell culture conditions, but also understanding how the tumor microenvironment, particularly the availability of nutrients, shapes cancer progression.

P-234	A comparison between male and female metabolomic profiles of hypertensive Black South Africans

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Introduction: Cardiovascular disease (CVD) is a growing concern in sub-Saharan African populations. Hypertension, a risk factor for CVD development, presents with great disparities in incidence between African and Caucasian populations. However, studies investigating the underlying mechanisms involved in hypertension development in Africans are scarce. Here we present data from the first untargeted metabolomics investigation done on Black South Africans, using a subset of the Sympathetic activity and Ambulatory Blood Pressure in Africans (SABPA) cohort. This cohort is exceptionally well phenotyped, including a series of life-style, anthropomorphic and biochemical measurements involved in CVD, and is thus very well suited for aiding in the understanding of hypertension development, which may lead to better, population specific treatment strategies. Methods: Male and female Black African participants were separately grouped into five quantiles (Q1-Q5) based on 24-hour sympathetic ambulatory blood pressure monitoring measurements. Metabolic profiling was done for only the lowest (normotensive) and highest (severely hypertensive) quantiles from each gender, using both gas- and liquid chromatography coupled mass spectrometry to capture a wide range of metabolites. Statistical analyses included t-tests and principle component analysis, resulting in VIP metabolite selection. Results: Despite comparable differences in males and females between Q1 and Q5 blood pressure measurements, African male participants presented with much more pronounced metabolic perturbations, when compared to their female counterparts. While male metabolic profiles clearly indicated disturbances in pathways influenced by the NADH/NAD+ ratio, this was not present in females, suggesting differences in mechanisms and risk factors involved in hypertension development, between gender groups.

P-235	Standardized Steroid-Profiling for Metabolic Phenotyping Research

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Steroid hormone profile analysis (mineralocorticoids, glucocorticoids, sex steroids) is of high interest for metabolic phenotyping and allows an improved understanding and role of the individual steroid hormones in metabolomics research. Steroid hormones are i.e. important endogenous metabolites in the cholesterol metabolism and are highly relevant for the microbiome and gender research. They have two principal biological functions: certain steroids (such as cholesterol) are important components of cell membranes which alter membrane fluidity, and many steroids are signalling molecules which activate steroid hormone receptors. UHPLC-MS/MS analysis is the technique of choice for multiplexed and quantitative steroid hormone analysis. We will present a standardized, ready-to-use steroid assay in kit-format intending the quantitative analysis of all 17 key steroids including automated analysis workflow and quality controlled data read-out. The steroid metabolites are testosterone, DHT, progesterone, cortisol, estradiol (E2), DHEAS, androstenedione, 17-OH, Progesterone, corticosterone, 11-Deoxycortisol, estrone (E1), DHEA, 11-Deoxycorticosterone, aldosterone, cortisone, etiocholanolone, androsterone. The UHPLC-MS/MS based assay includes standardized sample preparation in 96-well plate format. The sample preparation is performed by a solid phase extraction (SPE) procedure. Between 200 and 500µL serum sample volume is needed depending on the triple quadrupole mass spectrometry platform used. 7 minutes run time per sample allows a sample throughput of 82 samples in 20 hours. Scientific relevance of steroid hormones and assay performance data including inter-laboratory comparisons, ring trial and proficiency testing results demonstrating the precision, accuracy, stability, and reproducibility of the developed assay for human serum will be presented.

P-236	Determination of TCA Metabolites by Ion Chromatography HR/AM Mass Spectrometry

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Cancer, diabetes, and other metabolomics diseases are known to disrupt metabolomics cycles. [1] However, researching metabolomics pathways can be challenging. Many of the analytes are small ionic and structural isomers with the same fragmentation pattern, and therefore require chromatographic separation. Standard liquid chromatography methods have limited chromatographic resolving power for these types of metabolites. [2] Ion chromatography (IC), designed for the separation of ionic compounds, is ideal for the separation of ionic metabolites. Here we demonstrate IC as a complimentary separation technique by providing superior resolution and sensitivity of polar metabolites combined with the high resolution and accurate mass measurement (HR/AM) capabilities to differentiate isobaric metabolites. The anionic metabolites were separated by anion-exchange chromatography using a hydroxide gradient at 380 µL/min, optimized to resolve key phosphate isobars. The IC-MS interface was provided by methanol desolvation solvent and an electrolytic device desalting the eluent to water, making the eluent compatible with MS. The method was applied to head and neck cancer cell lines provided by UCLA School of Dentistry. Differential analysis was performed using Thermo Scientific™ Compound Discoverer™ software. Components of interest (p-value <0.05, fold change >2) were identified using high resolution accurate mass, MS/MS and retention time. The experiments showed that the aggressive cancer type cells displayed significantly higher levels of TCA metabolites than the less aggressive similar cell type.

P-237	NMR Metabolomics of body fluids as a potential predictive method of therapy outcome in HCC patients

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Hepatocellular carcinoma (HCC) is the second most common cause of cancer deaths worldwide. Late diagnostic and scarce therapy options are the main challenging factors in HCC healthcare. Sorafenib, a multikinase-inhibitor, is currently the only approved sytemic treatment of advanced HCC. However, therapy with Sorafenib only achieves a modest improvement in overall survival or delayed tumor progression. Thus, a systematic assessment method is urgently needed to identify primary or secondary therapy resistance to sorafenib. Therefore, we used NMR-based metabolomics to monitor tumor progression in comparison to routine clinical diagnostics. Using 1D- and 2D-NMR-based metabolomics, under standardized operating procedures, we analyzed body fluids, namely plasma and urine samples of advanced HCC patients (stage3, n=8), prior and following 4 weeks of systemic monotherapy with Sorafenib. Quantitative analysis of randomized processed samples of plasma lipoproteins and 25 compounds in urine using supervised and unsupervised methods were correlated with treatment outcome as evaluated by physicians according to clinical standards. Accordingly, analysis of lipoprotein levels in plasma clearly suggests a correlation between treatment response and cholesterol metabolism. Specifically, LDL and HDL particle size tightly correlate with patients' response status. Furthermore, unsupervised PCA analysis and metabolites profiling of urine yielded similar findings as the supervised lipoproteins results. Thus, classification matches with the clinicians' evaluation of Sorafenib treatment outcome. Hereby, NMR-based metabolomics of patients body fluids seem to correlate with therapy outcome by metabolomic reprogramming of the tumors non-invasively, and therefore provide a predictive classification, to potentially pave the way towards a personalized therapy evaluation of cancer patients.

P-238	Metabolomics and lipidomics as a research and diagnostic tool in inborn errors of metabolism

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Metabolomics and lipidomics are becoming increasingly important as analytical tools for biomarker discovery in research but have not frequently been applied to diagnostics. Inborn errors of metabolism are routinely identified by targeted analysis of intermediary metabolites. The growing development of high-resolution mass spectrometric techniques, however, opens new avenues to perform semi-targeted metabolomics and lipidomics in complex matrices. These matrices include plasma, cells and/or tissues from patients who suffer from known or unknown inborn errors of metabolism. In our study, we investigated the metabolome and lipidome profiles from a set of 120 plasma samples from patients with 10 known inborn errors of peroxisomal metabolism. We used reversed-phase, normal-phase, and hilic chromatography followed by full-scan orbitrap-MS on the Q Exactive plus in positive and negative ionization mode and annotated over 200 polar metabolites and over a 1000 lipid species. Furthermore, we identified specific biomarkers for the different inborn errors of peroxisomal metabolism and discovered new potential biomarkers. Because this is a semi-targeted approach, identities of some of the newly found biomarkers remain to be characterized. The ultimate goal is to use this type of metabolomics and lipidomics analyses for first line screening of inborn errors of metabolism.

P-239	Metabolic profiling of human brain tissue with Alzheimer's disease by UPLC-LTQ-Orbitrap/MS

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CO-AUTHORS: *Yoon Hwan Kim, Hoon Ryu, Kyoung Heon Kim, Bong Chul Chung*

Alzheimer's disease (AD) is a multifactorial chronic neurodegenerative disease which triggering progressive cognitive decline and memory loss. Even though AD was observed in 1900's, it has not yet been fully understood. Metabolomics studies could give us a new insight of view. In that sense, the aim of this study was to determine significant metabolites and pathways. We have compared metabolites in human brain tissue between AD and normal controls with an ultra-high-performance liquid chromatography coupled with linear ion trap-orbitrap mass spectrometer. Multivariate data analysis was performed by means of principal component analysis, projection to latent structures discriminant analysis and orthogonal projection to latent structures discriminant analysis. The result showed clear discrimination between AD and normal control groups. In positive electrospray ionization (ESI) mode, the most differential metabolites [a total of 59 (VIP scores > 1, p-value < 0.05) out of 1121 variable ions] included 2-oxoglutarate, phenylalanine, tyrosine originating from 2-oxocarboxylic acid metabolism, arginine and proline metabolism. In negative ESI mode, the most differential metabolites [a total of 12 (VIP scores > 1, p-value < 0.05) out of 114 variable ions] were cis-aconitate from citrate cycle and 2-oxocarboxylic acid metabolism, methylitaconate, 2,3-dimethylmaleate dopaguinone originating from nicotinate and nicotinamide metabolism, respectively.

P-240	Application of Quantitative Metabolomics in Disease Biomarker Studies

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Metabolomics has shown significant promise in the discovery of new biomarkers for a number of complex clinical disorders. The Metabolomics Innovation Centre (TMIC) has participated in several studies aimed at identifying predictive biomarkers for maternal and neonatal health. This work led to the identification of robust serum metabolite biomarkers for first-trimester prediction of early-onset pre-eclampsia, late-onset pre-eclampsia, Down syndrome and fetal congenital heart defects. Quantitative, multi-platform metabolomic analyses of maternal serum showed extensive group differences in the profiles of healthy expectant mothers and expectant mothers that later developed or were later shown to have the aforementioned neonatal/perinatal problems. Other studies conducted by TMIC have focused on finding diagnostic metabolite biomarkers for T cell–mediated rejection (TCMR) in pediatric kidney transplant recipients. Our findings suggest that quantitative urinary metabolomics can serve as a sensitive, specific and noninvasive tool for detecting TCMR and that this approach is superior to tissue biopsy and serum creatinine. These data also revealed that the biomarker profile exhibits very minimal overlap wiith other allograft injury processes. In veterinary applications, TMIC has identified predictive biomarkers for postpartum or periparturient diseases in dairy cattle. Using a multi-platform metabolomic approach, we showed that periparturient diseases such as metritis, mastitis, lameness and milk fever, can be predicted via serum metabolite profiles up to 6 weeks prior to their development. These biomarker profiles are being further validated and it is hoped that they will be translated to inexpensive bed-side or pen-side screening tests using portable devices that are being developed by TMIC.

P-241	Metabolomic profiling reveals BDE-47 could induce oxidative stress by inhibiting PPP pathway in MCF-7 cells

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CO-AUTHORS: *Li Xiang, Shangfu Li, Chunxue Yang, Zongwei Cai*

Polybrominated diphenyl ethers (PBDEs) were widely used as flame retardants for decades and gradually viewed as environmental contaminants because of its potential harmness to the organisms. Numerous researches have studied the connection of PBDEs to human health but few of them were related to breast carcinoma. In the present study, MCF-7 cells were exposed in vitro with different species of PBDEs (BDE-47, BDE-99, BDE-128, BDE-153) to study the influence to breast cells. Results showed that, BDE-47 was the most toxic one among the studied species and the toxicity to MCF-7 cells was gradually increased when the BDE-47 concentration exceeded 1 μM in the medium with 1% FBS. The following metabolomic study was conducted by ultra-performance liquid chromatography (UPLC) coupled with mass spectrometry (MS). It was found that pyrimidine metabolomic, purine metabolism and pentose phosphate pathway (PPP pathway) were significantly downregulated. The downregulation of PPP pathway would result to shortage of NADPH. The enzymes involved in reactive oxygen species (ROS) elimination system might be influenced because no sufficient NADPH could be used. Since the antioxidases could not clear up the ROS timely, oxidative stress occurred and caused damage to cells. Eventually, oxidative stress generated RNA damage and induced accelerating of RNA degradation. The pyrimidine metabolism and purine metabolism were downregulated by the degradation eventually. In conclusion, the BDE-47 could induce oxidative stress by inhibiting PPP pathway and disorder the whole metabolism of the whole cell subsequently.

P-242	Metabolomics Characteristics of Buyang Huanwu Decoction in Cerebral Ischemic Stroke Mice

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Background and Purpose?Buyang Huanwu Decoction (BHD), a TCM prescription, clinically has long been used for neurological recovery after stroke. In this study, NMR-based and QTOFMS metabolomics were performed to evaluate the therapeutic effect of BHD on the cerebral ischemic/reperfusion (CI/R) injury-induced sub-acute stroke mice. Methods?BHD was prepared as conventional method and lyophilyzied. An acute ischemic stroke in mouse model was induced by a middle cerebral ischemic/reperfusion (CI/R) injury. Brain tissue and cerebrospinal fluid (CSF) were used for the NMR-based and QTOFMS metabolomics studies. Results?Results of NMR data on brain tissue showed that BHD treatment amelioated the anaerobic glycolysis, alleviated the glutamate and GABA release from brain to periphery, and attenuated NAA release and taurine level. CSF metabolomics studies by NMR and QTOFMS revealed that hypoxanthine, inosine, dihydrouracil, D-threitol, inosine-5'-monophosphate disodium salt (IMP), malic acid, uridine, citric acid, AMP, succinate, N-acetylaspartylglutamic acid, phenylalanine, propionyl-L-carnitine, levulinic acid, glycerol 3-phosphate, deoxycytidine, 1-methyladenosine, pipecolic acid, cytidine, etc, significantly changed in CI/R-induced stroke. Furthermore, 10 endogenous metabolites, identified through 1H NMR analysis, were very different between CI/R-induced and sham groups. They are acetate, acetone, alanine, creatine, glucose, lactate, myo-inositol, N-nitrosodimethylamine, pyruvate, and succinate which demonstrated that metabolic changes in CI/R-induced stroke were also concerned with resultant shifts in energy supply and demand after BHD treatment. This finding provides a scientific evidence for the beneficial of BHD in TCM clinical application for ischemic stroke.

P-243	Global 1H-NMR Metabolomics of the Human Placenta over 24 hours

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The current data for optimization of placental specimen collection for metabolomics studies suggest immediate processing of specimens. This poses a significant challenge during specimen collection, and the lack of timely processing may result in loss of useful metabolic data. We evaluated changes in metabolites of placental tissue at various time-points up to 24 hrs post-delivery. Twelve gravid full-term, non-labored patients receiving Caesarian sections were consented for participation in an IRB-approved study at UF. Placental specimens were collected at 5 time-points:<5 min, 15 min, 30 min, 1 hr and 24 hrs. Nuclear magnetic resonance spectroscopy was conducted on a 600 MHz spectrometer to measure global metabolic profiles of placental samples. Significance of metabolites was determined using a Student's t-test of the area under the metabolite peak(s) of probabilistic quotient normalized spectra. While some metabolites (e.g. glucose, fructose, and lactate) were altered in the first 15 min following delivery, a pool of 20 was not significantly changed at 30 min. Metabolites significantly increased by 24 hrs included amino acids, ketones, TCA cycle intermediates, lactate, and sugars, while glycerophosphocholine, glucose and VLDL were significantly decreased (p<0.05). Several amino acids, creatine, and myo-inositol did not significantly change over 24 hrs. Our study challenges the current notion of immediate specimen processing, suggesting there may be valuable metabolic data present in tissue up to 30 min post-delivery. To date, there is limited data on metabolic transport in the placenta, and the persistence of metabolites may be used to gain information on altered metabolism in this tissue.

P-244	Effects of bioactive oligopeptides to human cells – a lipid- and metabolomics approach

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Effects of bioactive oligopeptides to human cells – a lipid- and metabolomics approach Introduction: Within the milk protein sequence, several bioactive peptides are encrypted which showed e.g. anti-inflammatory effects. For activation, an enzymatic digestion and purification is necessary. The anti-inflammatory bioactivity has been already published. However, the fundamental effects to the lipid- and metabolom has not been reported, yet. Therefore, this study aims to observe the changes of the lipid and metabolites profile in cells. Material and Methods: The peptides were used in an in-trans-approach. The cells were cultivated in calf serum and then in human serum. Within the serum cultivation, the treatments were conducted for 48 h. After preparation by the SIMPLEX approach, the cell extracts were analyzed by a 7T solariXR FT-ICR-MS (Bruker, Germany). The data validation was conducted by the QC protocol (Demetrowitsch et al., 2015). The data evaluations were conducted by non-targeted and targeted methods. For the non-targeted approach, a PCA and a classification algorithm were used to identify specify biomarkers. The lipidomics approach was conducted with the LipidXplorer. Results: The treatments vs. control showed tight clusters within the PCA model. The bucket statistic showed changed metabolite patterns for the high anti-inflammatory treatment. Changed concentration were observed for e.g. dityrosine and 6-chlorocatechine. The lipidomics approach provides e.g. highly significant changes for some phospholipids, like diacylglycerol (31:7) and phosphatidylinositol (44:3). These findings are in accordance with earlier studies and enable us to proceed with this cellular model system for mechanism clarification.

P-245	Metabolic phenotyping in the mouse model of urinary tract infection shows that 3-hydroxybutyrate in plasma is associated with infection

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Urinary tract infection (UTI) is one of the most common bacterial infections. Current diagnosis of UTI chiefly relies on its clinical presentation, urine dipstick tests and urine culture. Small molecule markers in blood for both infection and recovery would facilitate diagnosis and management of UTI. Mass spectrometry-based fingerprinting of plasma and urine at 3 time points, pre-infection (t = -24h), infection (t = 24h) and post 3-day treatment (t = 112h), were acquired in the following four groups: mice which were healthy, infected and not treated, infected and treated with ciprofloxacin and infected and treated with Relinqing Granules (n=6 per group). A metabolomics workflow including multivariate analysis and ROC regression was employed to select metabolic features that correlated with UTI and its treatment. Circa 4,000 molecular features were acquired for each sample. The small acid 3-hydroxybutyrate was found to be the most discriminatory metabolite for urinary tract infection in plasma, with an area under the curve = 0.97 (95% CI: 0.93-1.00, accuracy = 0.91, sensitivity = 0.92 and specificity = 0.91). The level of plasma 3-hydroxybutyrate was depleted after infection with a fold change of -22 (q < 0.0001). Correlation between plasma 3-hydroxybutyrate and urine bacterial number in all groups and time points was r = -0.753 (p < 0.0001). The findings show that plasma 3-hydroxybutyrate is depleted and strongly associated with UTI at both infection and post-treatment stages in a UTI mouse model. Further work is envisaged to assess the clinical potential of blood tests to assist UTI management.

P-246	Rapid, Comprehensive and Simultaneous Determination of Inborn Errors of Metabolism using a Validated Untargeted Metabolomics Methodology

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In-born errors of metabolism (IEMs) are inherited metabolic disorders. Most IEMs are caused by defects in the enzymes that help process nutrients, which result in an accumulation of toxic substances or a deficiency of substances needed for normal body function. Making a swift, accurate diagnosis of an IEM is critical in preventing brain damage, organ damage and even death. While there are several hundred recognized IEMs, state public health programs only screen for 40 disorders or fewer at birth. Currently, in order to accurately diagnose if and which IEM is present requires running an array of different targeted assays, which adds to costs and can ultimately only diagnose a limited number of IEMs. Metabolomic profiling gives us an astonishingly rich view of a patient's metabolic disturbances through a single test, suggesting that this technology has the potential to be an efficient, first-line phenotyping tool for the diagnosis and monitoring of IEMs. Here we present the use of the Metabolon untargeted metabolomics platform for the analysis of a discovery set of 200 pediatric plasma samples which included 21 known IEMs and 70 healthy individuals. The methodology correctly identified 20 of the 21 disorders in the panel that can be screened in blood plasma. In the clinic, many different biochemical tests would have been required to achieve a similar outcome. Importantly, this methodology accurately identified the affected individual patients when compared to a healthy population, demonstrating the power of this methodology for patient screening and personalized medicine.

P-247	Lipidomic evaluation of dog hair and skin samples – proof of concept study

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CO-AUTHORS: *Emma Bermingham, Nick Cave*

Skin and hair consist largely of proteins and lipids. It is thought that lipids affect parameters such as shine and feel, which are viewed by dog owners as important measures of coat quality, and by veterinarians as an indicator of dietary sufficiency, overall health, and wellbeing. However, to date coat quality is typically assessed by subjective measures which are difficult to replicate. Therefore, we aimed to understand whether skin and coat quality could be assessed quantitatively using lipidomics techniques. In order to understand the impact of collection method, hair and hair roots, skin swab with a cotton bud, and skin scrubbed with solvent, were collected from 3 dogs. Mass spectrometric analysis was carried out on a Q-Exactive Mass Spectrometer and non-targeted peak detection was undertaken using the R package XCMS. The data was analysed using the lipid identification software package LipidSearchTM. We identified 38 lipids, with 18 lipids in common across all four sample types. The two skin samples had a further 11 lipids in common, showing a higher number of ceramides and diglycerides compared to the hair samples. Lipid concentration varied between sample types, with skin samples showing larger amounts of Sphingolipids, than root and hair samples. Furthermore, skin samples show that lipids may be able to discriminate between individual dogs. Sampling method did not significantly affect number of lipids for each sample type, therefore enabling the use of methods kinder on the animal. Subsequently, patterns of hair and skin lipids need to be correlated with coat quality assessments.

P-248	Characterization of the fescue toxicosis metabolome in grazing beef cattle and the impact on it of different environmental conditions

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Plant-beneficial endophyte Epichloë coenophiala, infects tall fescue (Lolium arundinaceum), but also produces ergot alkaloids (EA) that are causative for fescue toxicosis (FT) in livestock. Multiple signs of FT align with metabolic perturbations and the disease is exacerbated by high temperature (T) and humidity (H). In three studies with Angus steers (n=12/study), animals were randomly assigned to toxic (E+) or non-toxic treatments (n=6/treatment); one study had sampling dates at different T and H, resulting in high or low T-H indices (THIs). Urine, blood, feces, weights and various physiological measurements were collected before and up to 28 days post pasture placement. Untargeted high-resolution metabolomics (HRM) was employed to analyze E+ grazing-induced plasma and urine metabolome changes and if these changes are influenced by variable THIs. From the data analyzed so far, more than 13,000 and close to 21,000 HRM features were detected in the, respectively, urine and plasma of the animals from the first study; the most significant effects were observed earlier in the urine and later in the plasma. Multiple EA were detected and their urinary and plasma profiles were different. In addition, metabolites indicative of tryptophan and lipid metabolism disruption caused by E+ consumption were observed. Some physiological effects of E+ grazing, i.e., increased respiration rates and rectal T were THI-independent, but the ear T was affected the most in E+ animals exposed to high THIs, suggesting that the effects of E+ on the plasma and urinary metabolomes might be affected by THI status.

P-249	Evaluating the Cardioprotective Effect and Compatible Principle of Xin-Ke-Shu formulae against Hypoxia/ Reoxygenation Injury in H9c2 Cardiomyocytes with Metabolomic Insights

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To date, no effective clinical therapy is available to treat ischemia/reperfusion (I/R) injury which is the major cause of myocardial cell death. Xin-Ke-Shu (XKS) is a traditional Chinese patent medicine used for treating coronary heart diseases. The objective of this analysis was to better decipher the possible molecular mechanism and compatible principle that XKS provides cardioprotection against I/R injury. Here, a hypoxia/reoxygenation (H/R) model in H9c2 cardiomyocytes was applied,,, to mimic the I/R injuries observed in vivo, and a cell metabolomics approach using the ultra performance liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (UPLC-Q/TOF MS) was conducted for the first time. The results of multiple evaluation indexes demonstrated the significant protective effects of XKS against oxidative stress and apoptosis induced by H/R injury. Multivariate statistical analysis showed that the metabolic profile of H/R group clearly deviated from the control group. Thirteen metabolites (C1-C13) were identified as potential biomarkers associated with H/R-induced injury, which were correlation with the aberrant pathways including sphingolipid metabolism, glycerophospholipid metabolism, fatty acid ?-oxidation metabolism and proteolysis. All these results indicated that the significant cardioprotective effects of XKS against H/R injury in vitro were probably comprehensive mediated by the suppression of oxidative stress, cell apoptosis, membrane disruption, mitochondrial oxidation and proteolysis. Our current data also demonstrated that Dan-Shen made crucial contribution to XKS in cardioprotective effects. This study may enable information from holistic cell metabolomics to be used for mechanism and compatibility rule elucidation of TCMs.

P-250	Human and tube variability are greater than storage variability in clinical metabolomics

PRESENTING AUTHOR: *Charmion Cruickshank-Quinn, University of Colorado Anschutz Medical Campus, United States*

CO-AUTHORS: *Laura Zheng, Kevin Quinn, Russell Bowler, Richard Reisdorph, Nichole Reisdorph*

Background: One major challenge in clinical metabolomics is lack of guidelines for sample collection and handling. Another challenge is the pace of the clinical environment that often does not allow for ideal sample collection conditions. These conditions include different tube types and the length of time samples sit following collection; both can confound results. Therefore we conducted metabolomics analysis of serum and plasma to determine the effect of time and tube type on the metabolome. Methods: Blood, from 3 individuals, was collected in Tiger, EDTA, and P100 vacutainers. Samples were stored at 4°C for 6 time points (0h, 0.5h, 1h, 2h, 4h, 24h) prior to centrifugation, aliquotting and storage at -80°C. Metabolomics was performed on plasma and serum using standard protocols. ANOVA with multiple testing correction and fold change > 2 compared (1) individuals at the control time point (0h), (2) tubes at 0h, and (3) time differences for each tube. T-tests compared each time point against 0h. Results: Individual person differences for the EDTA, P100, and Tiger tubes were 946, 1311, and 1205 respectively. Tube differences for individuals 1, 2, and 3 were, 887, 1069 and 655 respectively. Time differences for the EDTA, P100, and Tiger tubes were 43, 110, and 319 respectively. The greatest changes compared to the control time point, occurred at 1h for EDTA, 4h for P100, and 24h for Tiger. Conclusion: Our results suggest that subject-to-subject variation and tube-to-tube variation are greater compared to when samples are left on the benchtop.

P-251	Pesticide exposure in early pregnancy associated with fetal growth restriction

PRESENTING AUTHOR: *Aude-Claire MORILLON, INFANT Centre / University College Cork, Ireland*

CO-AUTHORS: *Shirish Yakkundi, Martin Wells, Philip Baker, Louise Kenny*

Fetal growth restriction (FGR) is defined as impaired fetal growth compared with the normal growth potential for the fetus. This pregnancy complication is estimated to affect 10% of pregnancies and leads to short and long-term complications for the baby. The use of pesticides is common practice in agriculture, and exposure during pregnancy has been linked with FGR, birth defects and impaired child neurodevelopment. This study assessed exposure to pesticides and their impact on fetal growth. A nested case-control study used urine samples collected at 20 weeks' gestation from pregnant women participating in the SCOPE study (www.scopestudy.net). Cases (n=20) were women with severe FGR (customised birthweight ? 5th centile, n=20) matched by age, body mass index and ethnicity to controls (n=20) who had uncomplicated pregnancies. Urine samples (100 µL) were diluted with MQ water (200 µL) before analysis. Data were collected for all samples in triplicate in untargeted positive ion mode using UPLC (BEH C18 column, 2.1x100 mm, 1.7 µm) coupled with mass spectrometry (Synapt G2-S, Waters), using a data independent analysis approach. The label-free data were normalised, processed and database searched using Progenesis QI (Nonlinear dynamics, UK). Data were searched against the Human Metabolite Database and pesticide library. Two pesticides (Kresoxim-methyl, Rabenzazol) were significantly increased (p <0.01) and 3 (Tolfenpyrad, Carbosulfan, Carbofuran) significantly lowered (p <0.05) in cases compared to controls. This study suggests that exposure to commonly used pesticides in early pregnancy may affect in utero growth trajectories. Further research is needed to clarify the precise association with FGR.

P-252	MALDI-MS-based High-throughput Metabolite Profiling of Human Body Fluids

PRESENTING AUTHOR: *Daisuke Miura, Kyushu University, Japan*

CO-AUTHORS: *Chihiro Kawano, Akiko Miki, Takanori Ishii, Yoshinori Fujimura, Hiroyuki Wariishi, Mitsuru shindo*

Introduction: Metabolite profiling is one of the represent techniques for obtaining information on the relationship between the metabolome and factors such as phenotype, quality, or bioactivity. Previously, we have reported that matrix-assisted laser desorption ionization-mass spectrometry (MALDI-MS) was useful for highly sensitive and high-throughput metabolite profiling. This technique become offline high-throughput MALDI-MS analysis combined with minimal operation of sample preparation. Herein, we applied this technique to analyze human serum and urine for high-throughput metabolite profiling and classification of normal, prostate and breast cancer specimens. Preliminary Results: Generally, it is known that there is no perfect MALDI matrix for ionizing all molecules, and ionizable molecular coverage of each matrix is limited. To obtain favorable MALDI matrix for detecting low-molecular-weight compounds from biofluids, we performed the matrix screening using more than 50 synthesized matrix library and commercially available matrix. As a result, 1,5-diaminonaphthalene (1,5-DAN) and synthesized matrix No. 19 (quinolone derivative) were found as favorable matrices for detecting several biofluid metabolites. We first investigated optimal sample preparation method (dilution rate of specimen, concentration of matrix, solvent of specimen and matrix). Then, we could find the best condition of sample preparation for high-throughput metabolite profiling of human biofluid by MALDI-MS. In this experiment, MALDI-MS analysis time is about 30 second and about 50 metabolite-derived peaks were obtained in a single run. The PCA score plots of obtained MS dataset from biofluids showed clear separation of clusters of healthy volunteers and diseases (breast and prostate cancer). Detailed results will be discussed in the session.

P-254	Serum metabolomic analysis for understanding estrogen-induced mammary tumorigenic mechanisms in female ACI/Seg rats

PRESENTING AUTHOR: *Yoshinori Okamoto, Meijo University, Japan*

CO-AUTHORS: *Shohei Ogiwara, Kanoko Sakata, Akira Aoki, Koji Ueda, Hideto Jinno*

Aim: Estrogen has been reported to be involved in mammary carcinogenesis due to its initiation and promotion effects. In addition, estrogen modulates a broad range of metabolic profiles, potentially leading to mammary tumorigenesis. In this study, we carried out serum metabolomic analysis in female ACI/Seg rats, an estrogen-induced mammary tumor model. Methods: All animal experiments were conducted in compliance with the Animal Experiment Guidelines of Meijo University. Female ACI/Seg rats (6-week-old; Harlan) were subcutaneously implanted with either 17?-estradiol (E2) pellet (2.5 mg E2/17.5 mg cholesterol) or control pellet (20 mg cholesterol). Serum was collected at 22 weeks after pellet insertion. Serum metabolomic analysis was carried out using QTOF mass spectrometer (Triple TOF 6600, AB Sciex) equipped with UHPLC (Shimadzu). Data were analyzed using open source database (XCMS and MS-DIAL). Results and discussion: All rats that received E2 pellet developed mammary tumor during this experiment, whereas no tumor was observed in the control group. E2 treatment significantly suppressed body weight gain; however, no significant difference in food consumption was seen between the two groups. These results suggest that E2 modulates the metabolic profile in ACI/Seg rats, and that these metabolic changes could be associated with mammary tumorigenesis. Indeed, serum metabolomic analysis detected 116 ions that were statistically different (p<0.01) between the groups. Upon analysis, it was found that some of these ions were phosphatidylcholines, lysophosphatidylcholines and sphingomyelins. Although further experiments are necessary, these lipid metabolites could be involved in mammary tumorigenesis in female ACI/Seg rats.

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P-255	Metabolic alteration in liver tissue following weight change by high fat diet

PRESENTING AUTHOR: *MIN-SUN KIM, Korea Basic Science Institute, South Korea*

CO-AUTHORS: *Geum-Sook Hwang*

Obesity is a health problem that is increasing all over the world. The repeated intentional weight losses followed by weight regain is known as yo-yo dieting. Weight change was also found to be associated with an increased disease risk for myocardial infarction, stroke, diabetes, and coronary heart disease. Although the potential harmful effects of weight change are recognized, the metabolism and the organic dysfunction associated with weight change remain unknown. Thus, the current study evaluated the metabolome of liver tissue and identified variations in the levels of significant metabolites in response to weight change. Metabolomic profiling of livers from mice was analyzed by 1H-NMR. Subsequently, quantification of important metabolite involved in weight change was investigated by GC/MS. Lipid profiling of mice was performed to reveal differences in the levels of specific molecular lipid species in the livers of weight change and weight gain phenotypes using UPLC-QTOF/MS. Among aqueous metabolites, alanine, glycine, isoleucine, and valine were decreased in weight changed mice compared with weight gain mice. These effect is assumed to be connected to upregulation of the gluconeogenesis in liver tissue after HF diet and to get enhanced in weight change mice. In lipidomic profiling of liver tissue, Cer and SM were a highly abundant lipids displaying significant changes in weight change vs weight gain group. Those sphingolipids play key roles in the regulation of functions of immune cells and might be changed by inflammation. Therefore, our finding indicate that obesity-induced hepatic inflammation can be enhanced by weight change.

P-256	Non-Targeted Screening of Extractables and Leachables in E-cigarettes using a Single Platform UPLC-APGC-QTOF-MS

PRESENTING AUTHOR: *Steve Wilson, Waters, Australia*

CO-AUTHORS: *Naren Meruva, Baiba Cabovska, Steven Lai, Dimple Shah, Kari Organtini, Gareth Cleland*

Both the GC and LC data was handled on a single data analysis platform. The potential candidate markers were screened against a known library of extractables and leachables compounds which was automatically interrogated using mass accurayc, isotopic fit and fragment matching. The structural elucidation of unknown contaminants present was performed by using elemental composition, database searching and fragment identifications via in-silico fragmentation of a downloaded structure compared to accurate mass fragment ion data. In this study, the various components of an e-cigarette (end caps, mouth piece, gauze, heating element and flavor formulation) were extracted separately and subjected to non-targeted high resolution screening using both UPLC and APGC analysis on a single QTOF-MS platform. Data was acquired using a DIA approach, consisting of alternating high and low collision energy states to generate precursor and fragment ions. The data from sample extracts were compared to reagent blank extracts to determine the differences and potential extractables. Ensuring these compounds do not pose any toxicological risks to the consumer relies on identifying the extractables. The UNIFI Scientific Information System utilises accurate mass precursor and fragment information to simplify data review and facilitate the decision-making process. It allows analysts to evaluate complex data in a more efficient way and quickly make decisions about the possible identity of known and unknown compounds. This application demonstrates how non-targeted screening using LC and GC workflows can be adopted on a single platform for extractable and leachable testing in food, cosmetics and pharmaceutical packaging applications.

P-258	Metabolic Markers for the progression of nonalcoholic fatty liver disease in Type 2 diabetes patients

PRESENTING AUTHOR: *Tao Wu, Center of Chinese Medical Therapy and Systems Biology, Shanghai University of Traditional Chinese Medicine, Shanghai, China, China*

CO-AUTHORS: *Ming Yang, Hanchen Xu, Lei Wang, Peiyong Zheng, Guang Ji*

The report aimed to identify metabolic markers for nonalcoholic fatty liver disease (NAFLD) development in type 2 diabetes mellitus (T2DM) patients. Using a targeted ultra performance liquid chromatography (UPLC) coupled with Triple Quadrupole mass spectrometry (TQ/MS), we compared serum bile acids levels in T2DM with NAFLD (n = 47) and age-gender matched T2DM without NAFLD (n = 47) for the first time. It is interesting that serum bile acid profiles in T2DM with NAFLD are greatly different from those without NAFLD, characterized by the significant elevation of TLCA, 3DHCA, GUDCA, CDCA24G, LCA and UDCA, which may be potential biomarkers for diagnosis of NAFLD in T2DM, and meanwhile it presents that those increased bile acids may participate the formation of NAFLD in T2DM patients. These findings suggest that these newly detected metabolites may participate in the progress of NAFLD in T2DM patients.

P-259	LC-MS based untargeted metabolomics to study biomarkers of aging in rat urine

PRESENTING AUTHOR: *ERHAN SIMSEK, Department of Chemistry, NUS, Singapore*

CO-AUTHORS: *Fong Yau Sam Li*

Aging is a complicated phenomenon involving gradual accumulation of detrimental changes in biological systems resulting in significant changes in cellular metabolism. Normal aging process also frequently accompanies numerous diseases such as Alzheimer's, diabetes and kidney disease. It has been proposed that aging process itself may be the underlying cause for these age-related diseases, and slowing down or reversing it can potentially prevent or cure them. To explore the metabolic changes involved in aging process, Sprague Dawley (SD) rats were chosen as model organism. Urine samples from healthy SD rats of 3 and 15 months were analyzed using UPLC-QTOF in ESI positive and ESI negative mode. Pooled quality control samples were also analyzed regularly throughout the run to check for system stability. After peak picking and statistical analysis, differential metabolites among different age 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 PCA and PLS-DA revealed separation among different age groups. Metabolic features responsible for the separation between groups were chosen based on their VIP values and univariate statistics. They were 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 pathways that could be implicated in aging process. The study provides new insights into the mechanism and biomarkers of aging process.

P-260	Mice Fecal Metabolites Analysis with LC-MS/MS

PRESENTING AUTHOR: *Takero Sakai, Shimadzu Corporation, Japan*

CO-AUTHORS: *Takanari Hattori, Shuichi Kawana, Yoshihiro Hayakawa, Eiichiro Fukusaki, Mitsuharu Matsumoto*

Intestinal microbiome plays an important role in health and/or disease because it influences pathological and normal homeostatic functions. Low-molecular-weight metabolites produced by intestinal microbiome are absorbed constantly from the intestinal lumen and carried to systemic circulation; they play a direct role in health and/or disease. We focused on mice feces and utilized LC-MS/MS. We evaluated 55 and 97 of metabolites with both ion-pairing method and non-ion-pairing method. In ion-pairing method, 17 of metabolites were detected in the extract of mice feces (peak area RSD <20%). Main compounds of 17 metabolites were amino acids. In non-ion-pairing method, 75 of metabolites were detected in the 10-fold diluted extract of mice feces (peak area RSD <20%). Main compounds of 75 metabolites were amino acids and nucleic acid-related substances. As an application to fecal metabolomics, feces derived from mice that the age was different (10-week-old and 70-week-old) were analyzed by non-ion-pairing method. As results of analyses for the extracts of the mice feces, 66 of metabolites were detected. The concentrations of 21 metabolites (e.g. adenine, aspartic acid, cholic acid, homocysteine, and thymidine) in aged mice (70-week-old) feces were obviously lower (p < 0.01) than those in young mice (3-week-old) feces. These differences seem to be caused by both the differences of intestinal microbiome and physical senescence. For example, the lower concentration of fecal cholic acid in aged mice depend on both the decrease of bacteria that catabolize conjugated bile acids and the reduction of the bile acid secretion by aging.

P-261	Alcohol use caused changes in the metabolite profile of first trimester serum samples of pregnant woman

PRESENTING AUTHOR: *Olli Kärkkäinen, University of Eastern Finland, Finland*

CO-AUTHORS: *Anni Lehikoinen, Marko Lehtonen, Seppo Auriola, Ulrich Tacke, Seppo Heinonen, Kati Hanhineva*

Our aim was to evaluate effects of alcohol use to the metabolic profile of women during the first trimester of pregnancy. We used serum collected during routine first trimester visit to the hospital from healthy controls (n=55), alcohol users (n=19), drug users (n=24) and tobacco smokers (n=40). We measured levels of metabolites with LC-qTOF-MS using HILIC column and positive ESI. Significant differences were found between the study groups in the metabolite profiles, such as alcohol and drug-using mothers had increased glutamate and decreased glutamine levels, and alcohol-using mothers had decreased serotonin levels when compared to the controls. If verified in future studies, these results could help to explain alcohol use caused damage to the fetus as well as detection of alcohol use during the first trimester of pregnancy.

P-262	jMorp: Japanese Multi Omics Reference Panel

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CO-AUTHORS: *Daisuke Saigusa, Seizo Koshiba, Yasutake Katoh, Matsuyuki Shirota, Shu Tadaka, Yuichi Aoki, Kengo Kinoshita, Masayuki Yamamoto*

It has been shown that biomolecules found in biomaterials whose concentrations vary among individuals reflecting disease risks of the subjects are useful and these are called disease biomarkers. Measurement of such biomarkers will provide valuable information for disease prevention and early diagnosis. Tohoku Medical Megabank Organization (ToMMo) has completed a multi-omics (metabolome and proteome) analysis of 1008 plasma samples that are collected from Japanese residents (male: 433, female: 575) who participated in the Tohoku Medical Megabank Project Community-Based Cohort Study. The data have been integrated into the database "Japanese Multi Omics Reference Panel (jMorp)" and is publicly available through online [https://jmorp.megabank.tohoku.ac.jp]. Metabolome data are measured by proton NMR and LC-MS, and proteome data are obtained by nanoLC-MS. In current state, we have released distributions of concentrations of 37 metabolites identified by NMR, distributions of peak intensities of 257 characterized metabolites by LC-MS, and observed frequencies of 256 abundant proteins (proteome data was measured with 501 plasma samples (male: 190, female: 311)). We are planning to update metabolome data with several thousands sample and add results of association studies among these molecules, genome, and individual's subjective data to our biobank.

P-263	Comprehensive metabolomics-based interpretation of the anti-obesity effects of Dolichos lablab (hyacinth bean) or milk thistle (Silybum marianum) administration in high-fat diet-fed mice

PRESENTING AUTHOR: *Dong Ho Suh, Konkuk University, South Korea*

CO-AUTHORS: *Eun Sung Jung, Seung-Hyung Kim, Digar Singh, Choong Hwan Lee*

Hyacinth bean (Dolichos lablab) is a lesser-known bean that exerts potential anti-obesity effects, but its mechanism and efficacy are not well-understood. In this study, we investigated the anti-obesity effects of hyacinth bean compared to milk thistle, which have been used for treatment of obesity-related diseases as traditional herbal plant, in high fat-diet (HFD) mice, along with their underlying mechanisms. C57BL/6J mice were orally administered hyacinth bean (25 mg/kg/day) or milk thistle (100 mg/kg/day) for 9 weeks along with HFD. The anti-obesity effects exerted by hyacinth bean and milk thistle on clinical parameters, suppression of weight gain and liver steatosis were similar, but with some different. Metabolic pathway analysis revealed that hyacinth bean or milk thistle administration mainly attenuated lipid, glucose, and bile acid metabolism-related metabolites changed by HFD, while hyacinth bean specifically attenuated most of amino acids derived from pyruvate. These potential biomarkers, such as lipids and amino acids showed high correlation with obesity-related blood parameters. From these results, we suggested that lower dose of hyacinth bean showed similar anti-obesity effects as milk thistle, confirmed by both clinical and metabolomics analysis.

P-264	Metabolomics Study on Plasma of Alternate Day Fasting and Exercise in Patients with Metabolic Syndrome using UHPLC-QTOF/MS

PRESENTING AUTHOR: *Bo Kyung Kim, Kyungpook National University, South Korea*

CO-AUTHORS: *Mi-Ri Gwon, Min Hee Kwon, Ji-Won Lee, Sook Jin Seong, Young-Ran Yoon*

Alternate Day Fasting (ADF; "feed day", alternated with 25% energy intake "fast day"), is known to be effective for weight loss, preventing and improving metabolic diseases. These effects are known to occur in normal weight but the influences of ADF on overweight and metabolic syndrome patients remain unclear. Therefore, we aimed to investigate the alterations of endogenous metabolites in metabolic syndrome patients after ADF. Thirty-six subjects with metabolic syndrome (BMI 23.1-39.3 kg/m2) were randomized into four groups: ADF and exercise (Ex, aerobic and muscle exercise); only ADF; only exercise group; and a control group, for 8 weeks. Plasma samples were collected before and after clinical trial and analyzed by an ultra-high performance liquid chromatography (UHPLC)-quadrupole time-of-flight (QTOF)/mass spectrometry (MS) and multivariate data analysis. Principal component analysis (PCA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) score plots showed a clear separation between 4 groups. These results suggest that ADF and exercise are effective in the prevention and improvement of weight loss and overweight in the metabolic syndrome patients, then to reach solid conclusion in this research, the larger sample size and more strict clinical trials are required. Also, this study represents the first UHPLC-QTOF/MS-based metabolomics study to evaluate the influence of ADF and exercise on the metabolite alterations in overweight and metabolic syndrome patients. Therefore, UHPLC-QTOF/MS-based metabolomics research can be a useful tool for evaluating the effects of dietary and exercise controlling and confirming the metabolic syndrome mechanism.

P-265	Using GC-MS-based metabolite profiling to predict surgical success in otitis media

PRESENTING AUTHOR: *Hayley Abbiss, Murdoch University, Australia*

CO-AUTHORS: *Joel Gummer, Robert Trengove, Andrew Currie, Ruth Thornton*

Otitis Media (OM-middle ear infections) are the most common reason a child will visit their GP and be prescribed antibiotics. OM is usually associated with the build-up of middle ear fluid (MEF), which is a common cause of conductive hearing loss. In children with persistent or recurrent OM, surgical insertion of grommets to allow ventilation of the middle ear is often performed; however, in a third of children, disease recurs and repeat surgery is required. The aim of this study was to identify sensitive metabolomic biomarkers from MEF and saliva samples associated with surgical success. MEF and saliva samples were collected from 60 study participants prior to grommet insertion. Of these, 14 required repeat surgery. To determine the appropriate amount of sample to extract and analyse, preliminary analyses were performed on a pooled MEF sample. Serial dilutions of a 50 µL pooled sample were prepared giving equivalents of 0.625 – 10 µL of sample. Metabolites were extracted with water and methanol and derivatised for GC-q-TOF MS analysis. MS data showed that the MEF samples were rich with features even with the analysis of very small amounts of fluid. Chromatographic overloading and detector saturation of some features was evident even in the lowest sample volume; however, the response of most features in overloaded and saturated regions was linear up to 5 µL of sample. Samples will be extracted and derivatised using the equivalent of 5 µL of sample to uncover whether sensitive biomarkers of surgical success can be determined.

P-266	Targeted and non-targeted metabolomics to study developmental neurotoxicity of biocides

PRESENTING AUTHOR: *Jonathan Moss, Bruker Pty Ltd (Australia) - Bruker Corporation, Australia*

CO-AUTHORS: *Pim Leonards, Heiko Neuweger, Jonathan Moss, Sven Meyer, Aiko Barsch*

Worldwide, serious concern has arisen about the increased incidence of learning and developmental disorders in children. The European DENAMIC project investigated the behavior and cognitive effects of pesticide exposure in mice and rats and studied underlying molecular mechanisms of the observed effects using metabolomics. The current paper will focus on a targeted and untargeted metabolomics approach using one software package. Mice and rats were exposed to individual pesticides such as chlorpyrifos, carbaryl, cypermethrin and endosulfan. Behavior effects were studied once the animals reached adulthood. Metabolomic analysis was carried out using brain tissues of mice and rats. Data for targeted and untargeted metabolite analysis was acquired by HILIC-QTOF-MS/MS. MetaboScape3.0 software was used for data processing including mass recalibration, multi-pass recursive feature extraction with de-isotoping, deconvolution of adducts, and retention time alignment. Identification of known metabolites was based on retention time, accurate mass, isotopic pattern information, and MS/MS spectra. A custom made analyte target list of about 700 metabolites for annotation of known compounds, followed by automatic annotation of unknown peaks was used. Annotated compounds, and calculated elemental composition of unknowns, were further evaluated in an interactive list. The list interacts with box-whisker, PCA, and volcano plots providing direct information on differences between treatments and pointing to characteristic compounds for further investigation. Tentative identification of unknowns with associated MS/MS spectra was conducted by in-silico fragmentation based on the MetFrag algorithm. Data analysis time was reduced due to simultaneous and confident assignment of known targets and tentative annotation of unknown peaks.

P-267	Metabonomic difference between orthotopic and subcutaneous xenograft of pancreatic cancer: Identification, causes and notions

PRESENTING AUTHOR: *Jianghua Feng, Xiamen University, China*

CO-AUTHORS: *Bohan Zhan, Shi Wen, Heguang Huang*

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal tumors. However, the methodological differences between orthotopic and subcutaneous xenograft (OX and SX) models will cause confusion in understanding its pathological mechanism and clinical relevance. In this study, SX and OX models were established by implanting Panc-1 and BxPC-3 cell strains under skin and on the pancreas of mice, respectively. The tumor tissue and serum samples were collected for detection of 1H NMR spectroscopy followed by univariate and multivariate statistical analyses. As results, no obvious metabonomic difference was demonstrated in serum between the two models, however, the model- and cell strain-specific metabonomic differences were observed in tumor tissues. According to the KEGG analysis, ABC transporters, glycerophospholipid metabolism, purine metabolism and central carbon metabolism were identified to be the most significant components involved in metabonomic differences. Considering the methodological discrepancy in SX and OX models, such differences should be contributed to tumor microenvironment. In general, SX are not equivalent to OX models at molecular level. Subcutaneous transplantation displayed its inherent limitations though it offered a simple, inexpensive, reproducible and quantifiable advantage. And orthotopic transplantation may be favorable to simulate PDAC in patients due to its similar pathogenesis to human pancreatic cancer.

P-268	Metabolomic profile of exosomes released by FaDu cells exposed to ionizing radiation

PRESENTING AUTHOR: *Lukasz Marczak, Institute of Bioorganic Chemistry Polish Academy of Sciences, Poland*

CO-AUTHORS: *Agata Abramowicz, Anna Wojakowska, Piotr Widlak, Monika Pietrowska*

Exosomes are membrane-derived nanovesicles of 30-120 nm in diameter, actively secreted by various types of normal and cancer cells. Numerous studies have identified exosomes as a means of intracellular communication. The composition of exosomes differs from a cell type to a cell type. More than 41 000 different proteins have been reported as cargo of exosomes and are listed in databases like Exocarta (http://www.exocarta.org) and Visclepedia (http://www.microvesicles.org). In addition to proteins, exosomes contain lipids, particularly raft-lipids like ceramides, sphingolipids, cholesterol and glycerophospolipids. Beside them, bioactive lipids, such as prostaglandins and leukotrienes, and enzymes activated in lipids metabolism, such as phospholipase C are also found in exosomes. Although the lipid content in exosomes is extensively studied, changes in lipid profile after ionizing radiation have not yet been fully understood. In our study we used FaDu cell line as an experimental model. The FaDu line was established from a punch biopsy of an hypopharyngeal tumor. Cells were cultured in a medium with exosome depleted serum (Exo-) and irradiated using a therapeutic linear accelerator (Clinac 600) at 2 and 8 Gy/min dose rate. After 24 hours of irradiation the medium was collected and exosomes were isolated using a protocol which we developed. Both exosomes and cells were analyzed. For analysis of total metabolites, GC/MS techniques were applied, and for lipid profiling shotgun direct analysis Advion Nanomate source and Orbitrap MS were used. A. Abramowicz and L. Marczak equally contributed to this work. Project was supported by the National Science Centre, Poland, Grant 2013/11/B/NZ7/01512.

P-269	Lipidomics comparing DCD and DBD liver allografts uncovers lysophospholipids elevated in recipients undergoing early allograft dysfunction

PRESENTING AUTHOR: *Jin Xu, King's College London, United Kingdom*

CO-AUTHORS: *Ana Casas-Ferreira, Cristina Legido-Quigley, Yun Ma, Arundhuti Sen, Min Kim, Petroula Proitsi, Maltina Shkodra, Maria Tena, Parthi Srinivasan, Nigel Heaton, Wayel Jassem*

Background. Finding specific biomarkers of liver damage in clinical evaluations could increase the pool of available organs for transplantation. Lipids are key regulators in cell necrosis and hence this study hypothesised that lipid levels could be altered in organs suffering severe ischemia. Methods. Matched pre- and post-transplant biopsies from donation after circulatory death (DCD, n = 36, mean warm ischemia time = 21min) and donation after brain death (DBD, n = 76, warm ischemia time = none) were collected. Lipidomic discovery and multivariate analysis (MVA) were applied. Afterwards, univariate analysis and clinical associations were conducted for selected lipids differentiating between these two groups. Results. Matched pre- and post-transplant biopsies from donation after circulatory death (DCD, n = 36, mean warm ischemia time = 21min) and donation after brain death (DBD, n = 76, warm ischemia time = none) were collected. Lipidomic discovery and multivariate analysis (MVA) were applied. Afterwards, univariate analysis and clinical associations were conducted for selected lipids differentiating between these two groups. Conclusions. These findings suggest that LysoPC (16:0) and LysoPC (18:0) might have a role in signalling liver tissue damage due to warm ischemia before transplantation.

P-270	A new metabolic approach to treat patients with drug resistant epilepsy

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CO-AUTHORS: *Jack Germaine, Neha Kaul, Terence O'Brien*

Triheptanoin, the triglyceride of heptanoate, is being developed for several congenital metabolic deficiencies to provide energy. Heptanoate is metabolised directly into succinyl-CoA and is therefore anaplerotic. Our randomised, double blinded, placebo-controlled trial assessed the safety and tolerability of add on oral triheptanoin in patients with drug resistant epilepsy for 12-weeks of treatment. Patients were randomised for triheptanoin or "standard" medium chain triglycerides (MCT, triglycerides of octanoate and decanoate) to the maximum tolerated dose (maximum 35% of caloric intake). 18 patients were randomised to treatment with triheptanoin and 17 to MCT. There were no differences between treatment and MCT in the following parameters: 1) the number of patients finishing the full study (10 vs. 11 patients; 56% vs. 65%), 2) the time until withdrawal, 3) the number of adverse events and 4) the percentage of prescribed treatment dose taken over the treatment period (85% vs. 98%). Moreover, there were no changes between the treatment and placebo group in caloric intake over the treatment period (p=0.32), body weight changes (p=0.93) or amount of triheptanoin (0.59 +/- 0.28 g/kg) or MCT (0.72 +/- 0.24 g/kg, p=0.119, rank sum) taken per body weight per day (mean +/- SD). The study was not powered to investigate effects on seizure frequency, however five patients on placebo and one patient on triheptanoin showed >50% seizure reduction. In summary, in terms of safety and tolerability there was no difference between MCT and triheptanoin treatments when added to a normal diet for adults with treatment resistant epilepsy.

P-271	Inhibitory effect of Selaginellins from Selaginella tamarscina (Beauv.) Spring against cytochrome P450 isoforms on human liver microsomes.

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CO-AUTHORS: *Jae Kyung Heo, Zhexue Wu, Kwang-Hyeon Liu*

Selaginella tamariscina (Beauv.) has been used for traditional herbal medicine in not only Korea but also Asia especially in China. The ingredients of Selaginella tamariscina (Beauv.) has been also known to have various biological activities including anti-cancer activities. In this study, we evaluated the drug-drug interaction potential of four selaginellins obtained from Selaginella tamariscina against ten cytochrome P450 isoforms (CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP2J2, and CYP3A) in human liver microsomes using cocktail incubation and tandem mass spectrometry. They showed moderate inhibitory potential on CYP2C8, CYP2C9 and CYP2J2 enzyme activities (IC50 < 5 ?M), whereas they had weak inhibitory activities against CYP2A6 and CYP2E1 enzymes (IC50 > 20 ?M). Among four compounds tested, one compounds showed more weak inhibitory potential against 10 P450s than other three compounds. This work was supported by the Bio-Synergy Research Project (NRF-2014M3A9C4066462) of the Ministry of Science, ICT, and Future Planning through the National Research Foundation of Korea (NRF).

P-272	Metabolic cellular response towards immunotoxic compounds in Jurkat T cells

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A variety of natural products from microbes, plants, or other sources have been shown to influence the human immune system. Immunotoxicity of xenobiotics is up to now mainly investigated by in vivo experiments using laboratory animals. In this in vitro study the metabolic response of Jurkat cells after treatment with immune modulating natural compounds was analyzed using the cell line as a model for T cells of the human immune system. The aim was to discover whether investigations of the metabolome could contribute to the prediction of immunotoxicity. Data analysis included extracellular metabolome investigations by 1H-NMR spectroscopy. Amino acids were analyzed by LC-MS/MS, quantification of nucleotides was done using an ion-pairing HPLC method. Polar intracellular metabolites of the central carbon metabolism were investigated by GC-MS methods. Seven immune modulating natural products investigated at IC25 and three nontoxic controls were selected. Effects on mean metabolic pathways of Jurkat cells were found to be very specific for each compound. Concerning the metabolism of amino acids the level of four amino acids were significantly increased after cell treatment with all immune modulating compounds compared to untreated cells and also increased compared to cells treated with nontoxic controls. In summary the metabolomic studies of the Jurkat T cell line were used to identify potential metabolic biomarkers for prediction of immunotoxicity.

P-273	Simultaneous Metabolic Profiling in Colorectal Cancer Using Seemingly Unrelated Regression

PRESENTING AUTHOR: *Min Zhang, Purdue University, United States*

CO-AUTHORS: *Chen Chen, Nagana Gowda, Dabao Zhang, Daniel Raftery*

Colorectal cancer (CRC) is one of the most prevalent diseases worldwide and considerable effort has been devoted to identifying robust biomarkers to enable prediction, screening and surveillance. To understand the differences in metabolite levels between CRC and polyp patients as well as healthy controls, we employed seemingly unrelated regression (SUR) to model a group of metabolites simultaneously while adjusting other clinical covariates including gender, BMI, age, alcohol use, and smoking status. In addition, we used ParCorA algorithm to construct metabolite networks in CRC, polyp patients, and healthy controls, respectively, for all groups of metabolites both before and after using SUR to account for the effect of confounding variables. Results showed that metabolite levels were significantly affected by clinical covariates such as gender, BMI, and smoking status. While several metabolites show significant difference between CRC, polyp patients and healthy controls, SUR models also identified a number of significantly altered associations for groups of biologically connected metabolites. Furthermore, different metabolite networks constructed in CRC, polyp patients and healthy controls are different for several groups of biologically related metabolites before and after SUR analysis. Our results provide new insight into addressing the large confounding effects in the metabolomics profiling and shed light on establishing reliable noninvasive biomarkers for various types of diseases.

P-274	Importance of amino acid Citrulline as a metabolomic signature in determining the gut enterocyte status in Celiac disease

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CO-AUTHORS: *Somesh Kumar, Sunil Polipalli, Seema Kapoor*

BACKGROUND:Because the endogenous source of amino acid citrulline is small intestine and doesn't take part in protein,this study was designed to evaluate citrulline levels in patients with CD & in their FDR & to establish a correlation between histopathological findings &the citrulline levels as metabolomic for villous atrophy METHODS:The procedure adopted for measuring citrulline on DBS filter paper was TMS(LC-MSMS)& plasma citrulline was analysed by RP-HPLC.The disease state was confirmed by HLA typing RESULTS: Plasma citrulline value in 54 serology positive subjects was 9.0umol/L,in124serology negative subjects(FDR)was 24.3umol/L with pvalue of <0.0001.Correlations between biopsy grades of Subjects with their citrulline levels were established.For Marsh 3c grade lesions,citrulline levels were 5.6umol/L,for Marsh 3b,levels were 15.0umol/L with p value 0.006. Understandably the patients with total villous atrophy had a lower citrulline levels even if they were asymptomatic.All the patients were on stringent six month follow up on GFD &the value after 6months was 11.53umol/L DISCUSSION:Citrulline may serve as a sensitive Non-HLA marker of severity of intestinal damage.It is simple,minimally invasive and promising metabolomic tool which suggests the improvement in gut enterocyte mass in patient on GFD from the remotest area of the country by using DBS filter paper. Evaluating citrulline as a metabolomic and proteomic signature plays a very crucial role in the detection of silent and potential phase of the disease in the highest risk group like FDR.This novel finding will give us valuable information on metabolic pathway underlying disease pathogenesis,thereby providing us with tools to create novel diagnostics and therapeutics in the future

P-276	Identification of metabolite profiles from germinated soy germ extracts and improvement effects of their female menopause symptoms

PRESENTING AUTHOR: *Woo Duck Seo, National Institute of Crop Science, RDA, South Korea*

CO-AUTHORS:

This research was the first to investigate changes in metabolite profiles of germinated soy germ extracts(GSGE). Thirteen isoflavones and ten soyasaponins were characterised as aglycone isoflavones (genistein etc.), glycosylated isoflvones (genistin etc.), oleanane triterpenoid glycosides (soyasaponins Ab etc.) using ultra performance liquid chromatography quadrupole time-of-flight mass spectrometry (UPLC-qTOF-MS) and nuclear magnetic resonance (NMR). Especially, soyasaponin Ab and soyasaponin Bb from germinated soy germ were the predominant constituents (40-45 %) with significant differences in cultivars and germinated times. Also, we investigated on the prevention of osteoporosis, which are typical disorders of menopausal women. The GSGE was regulated which is enriched with soyasaponins osteoblasts via the bone morphogenic protein-2 (BMP-2) stimulated induction of alkaline phosphatase (ALP). The osteogenic activity of GSGE was accompanied by synergistic induction of Runx2 as the master transcription factors for bone formation. In conclusion, we suggest that anti-osteoporotic activity of GSGE could be because of its osteoblast activity, and it could be developed as a potent functional food and pharmacological agent for bone health.

P-277	Analysis of plasma samples for identification of schizophrenia biomarkers

PRESENTING AUTHOR: *Simon Ovenden, DST Group, Australia*

CO-AUTHORS: *Chad Bousman, Christos Pantelis, Ian Everill*

Schizophrenia is a severe mental disorder that affects approximately 1% of the global population.[1-2] There are several common symptoms that characterise schizophrenia including delusions and hallucinations, dysfunction and altered cognitive behaviour. It is thought that the onset of schizophrenia is caused by either or both of genetic makeup and environmental factors. Several classes of metabolites, including lipids and organic and amino acids, have been identified as potential biomarkers for schizophrenia.[1-4] As part of an ongoing program looking at identifying biomarkers for mental illness, plasma from a cohort of schizophrenia patients was analysed. Plasma samples were prepared for metabolomic and lipidomic sample analysis by both high resolution LC-MS and 1H NMR. Putative biomarkers were identified and confirmed through online database searches and additional chemical analysis. This presentation will discuss the current results of this ongoing research project, including sample preparation and analysis, multivariate statistical analysis results and biomarker identification. [1] Ying Qiao et al. Plasma metabolomics study of first-episode schizophrenia treated with olanzapine in female patients. Neuroscience Letters, 2016, 617, 270-276. [2] Y. He et al. Schizophrenia shows a unique metabolomics signature in plasma. Translational Psychiatry, 2012, 2, e149. [3] Matej Oreši? et al. Metabolome in schizophrenia and other psychotic disorders: a general population-based study. Genome Medicine, 2011, 3:19. [4] Matej Oreši? et al. Phospholipids and insulin resistance in psychosis: a lipidomics study of twin pairs discordant for schizophrenia. Genome Medicine, 2012, 4:1.

P-278	Metabolomics applied in skeletal muscle impact trauma

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CO-AUTHORS:

Muscle injury is a prevalent cause of debilitation for workers, athletes, and the public generally at home and in motor vehicle accidents. To date, a number of physical trauma models have been established, including in vivo and in vitro models, which lay a foundation of research into skeletal muscle injury. While the general processes associated with skeletal muscle injury have been described, some of the more specific factors involved in the recovery processes are identified based on experimental research as well. The project will apply global metabolomics based approaches in in vivo model. Specifically, a rat impact contusion model will be utilised to model impact trauma. Dynamic global metabolite profiling using GC-MS/MS will be performed at 6h, 12h, 1, 3, 7 and 14 days on tissue homogenates to identify factors that are associated with the initial recovery response following injury. There are temporal changes to biochemical pathways and processes during recovery from muscle trauma in vivo, and global metabolomics profiling will reveal these changes. Dominant endogenous factors present in injured muscle will activate signalling pathways resulting in secretion of cytokines that regulate inflammation and regeneration in skeletal muscle.

P-279	Short-term effect of wasabi rhizome (Wasabia japonica) on metabolic syndrome in high-fructose-diet-fed rats

PRESENTING AUTHOR: *Fernanda Thomaz, University of Queensland, Australia*

CO-AUTHORS: *Sunil Panchal, Lindsay Brown, Leigh Ward, Simon Worrall*

The prevalence of metabolic syndrome (MetS), a group of metabolic dysfunctions associated with obesity, has reached over 30% worldwide. Plant products such as wasabi rhizome appear to be effective therapeutic agents against obesity and chronic diseases. This study aims to investigate the short-term effects of Wasabia japonica rhizome on the metabolic syndrome induced in Wistar rats by a high-fructose diet. Methods: Rats were fed either a corn starch (C) or high-fat high-carbohydrate (H) diet for 8 weeks. After 8 weeks, CW (corn starch plus wasabi; n=12) and HW (high-fat high-carbohydrate plus wasabi; n=12) rats had 5% (w/w) wasabi rhizome added to their diet for 8 weeks. Animals had ad libitum access to food and water. Metabolic parameters including body mass index (BMI), systolic blood pressure (SBP), together with plasma cholesterol, AST and ALT, and hepatic histology were measured after the 16-week protocol. Results: Animals in the CW and HW groups had their BMI reduced by 13% and 17%, along with declines in SBP of 3mmHg and 14mmHg, when compared to controls. Plasma ALT and AST of the CW and HW groups were also significantly decreased when compared to controls, whereas total cholesterol was lower in the HW, but not in the CW group. Furthermore, hepatic histology showed less inflammation in the wasabi-treated groups. Conclusions: This 16-week trial suggests that wasabi exerted an ameliorative effect on the studied metabolic parameters, especially on body weight, SBP and inflammatory parameters. Further studies are required to investigate the molecular mechanisms underlying our findings.

P-280	Metabolomics facilitated the characterization of functional small molecules towards a diversity of biological innovations

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CO-AUTHORS:

In vivo small molecules as necessary intermediates are involved in numerous critical metabolic pathways and associated with many essential biological functions. In recent years, there is growing evidence to manifest that metabolomics is emerging as a powerful tool for facilitating the discovery of functional small molecules which provides novel insights into a diversity of biological concerns involving disease diagnosis, therapeutic discovery and toxicology. Yet, metabolomics holds promise in promoting widely biological innovations in a real world besides terming biomedical niche, which also yields a wealth of information about food and environmental questions concerning safety and toxicology. In this proposed presentation, brief introduction to metabolomics science shall be given to highlight its concept, protocol, manipulation and biological application for the first instance, and then a line of typical examples will be adopted to demonstrate how metabolomics combining with genetic strategy was employed by my group to facilitate the discovery of functional small molecules whose pattern changes can efficiently delineates the complex mechanisms implicated in a diversity of biological events via targeting the most affected metabolic pathways, such as disease progression, pathogen virulence and discovery and validation of therapeutic targets as well.

P-281	NMR-based metabolomic analysis for the diagnosis of systemic sclerosis

PRESENTING AUTHOR: *Sehyun Oh, Seoul National University, South Korea*

CO-AUTHORS: *Kyong-Hee Jung*

Systemic sclerosis (SSc) is a complex multi-organ autoimmune disease that is caused by inflammation, vasculopathy and fibrosis. Clinical heterogeneity, unpredictable course, high mortality and resistance to treatment make physicians still frustrated. To enhance diagnostic ability, new classification criteria of SSc is published by ACR/EULAR in 2015. However, no disease specific biomarker for SSc is present yet. Recently, there are unmet needs of useful biomarkers for diagnosis, evaluation of disease activity and severity in SSc. Metabolomics is expected to be a useful tool for the identification of biomarkers and new therapeutic targets. Metabolomic analysis has been reported for major rheumatic diseases, such as systemic lupus erythematosus, and rheumatoid arthritis, although none yet for SSc.

P-282	Radish Extract Affects the Gut Microbiome in Ovariectomy-induced Mice

PRESENTING AUTHOR: *yujin hwang, National Academy of Agricultural Science, RDA, South Korea*

CO-AUTHORS: *Hana Yi, Min-Jung Shin, Hwan-Hee Jang, Jung-Bong Kim, Jeong-Sook Choe*

The radish (Raphanus sativus) is an edible root vegetable of the Brassicaceae family. Radishes are grown and consumed throughout the world, being mostly eaten raw as a crunchy salad vegetable. Radishes are rich in ascorbic acid, folic acid, and potassium. They are a good source of vitamin B6, riboflavin, magnesium, copper, and calcium. However, little is known about the change of gut microbiome by radish. The gut microbiome was analyzed using the 16S rRNA gene sequences. The beta-diversity analyses revealed that the overall composition of gut microbiome of mice changed by radish. In addition, Radish changed the gut microbiota structure characteristically as a reducing Firmicutes phylum, especially Lactobacillus, and a rising Verrucomicrobia phylum. And we also were identified as Akkermansia muciniphila at the species level. The results were concluded that Radish could positively affect gut flora structure. However, we have to more study to find the link between the bacterial members and the host metabolism.

Name Last, First	Abstract
* = Presenting Author	
Abbiss, Chris	O-356
Abbiss, Hayley	P-265*
Abbott, Derek	P-17 P-47
Abbott, Sarah	P-7
Abdelnur, Patrícia	O-477*
Abell, Guy	O-178
Abramowicz, Agata	P-268
Acosta Sanchez, Abel	O-77
Adamski, Jerzy	P-175
Addison, Daniel	P-286
Adel-Patient, Karine	P-145
Agellon, Luis	O-352
Aharoni, Aharoni	O-106
Akarasereenont, Pravit	P-83
Aksenov, Alexander	O-479
Albanes, Demetrius	P-221
Albertson, Mark	P-236*
Alexandrov, Theodore	O-268 O-273 O-479
Alharbi, Omar	O-502
Ali, Ashraf	P-62*
Aliani, Michel	P-161*
Allard, Pierre-Marie	O-263
Almeida, João Ricardo	O-477
Alonso, David	P-45 P-49
Alqarni, Ayedh	P-7
Altadil, Tatiana	O-203
Amati-Bonneau, Patrizia	P-196
Ametaj, Burim	P-240
Amminger, Paul	O-134
Ammons, Mary Cloud	O-324
Amorós, María	P-86
Anacleto, Joe	P-76*
Andersson, Erik	P-107
Andersson, Lars	P-30
Andres, Christian	O-300
Antalick, Guillaume	O-128
ANTONIO, CARLA	P-52*
Aoki, Akira	P-254
Aoki, Masahiro	P-6
Aoki, Yuichi	P-262

Name Last, First	Abstract
* = Presenting Author	
Aow Shao Bing, Johanan	O-388
Apers, Sandra	P-19
Apffel, Alex	P-47
Apffel, James	P-17*
Apostolides, Zeno	O-123
Arita, Masanori	O-264
Arndt, David	P-3
Arnold, Jonathan	P-94
Aros, Sandrine	O-304 P-43
Arroyo, Vicente	O-168
Artati, Anna	P-175
Aru, Violetta	P-24*
Ashrafian, Hutan	O-474
Ashtiani, Samira	P-44
Asikin, Yonathan	O-93* P-153
Assempour, Nazanin	P-2
Athanasiou, Thanos	O-474
Atzori, Luigi	O-425
Aura, Anna-Marja	O-461
Auray-Blais, Christiane	P-173* P-189
Aurich, Maike	O-333*
Auriola, Seppo	O-289 P-261
Avery, Vicky	O-157
Aw, Wanping	P-190
Ayson, Marites	P-47
Bachman, Martin	P-284 P-286
Bacic, Antony	O-273
Baell, Jonathan	P-70
Bahado-Singh, Ray	P-168 P-240
Bajdzienko, Krzysztof	P-110
Baker, Erin	O-104
Baker, Jarrad	P-120
Baker, Philip	O-341 O-342 P-184 P-251
Baker, Wendy	O-197
Bakke, Per	O-179
Baldwin, T	P-108
Balog, Julia	O-146

Name Last, First	Abstract
* = Presenting Author	
Bamba, Takeshi	P-28* P-59 P-90 P-137
Banerjee, Rintu	P-212
Bansal, Aruna	O-179
Bararpour, Nasim	O-62*
Barbas, Coral	P-204
BARBIER SAINT HILAIRE, Pierre	O-319*
Baribaud, Frederic	O-179
Barlow, Christopher	O-436
Barrios, Clara	O-139
Barrios Barrera, Clara	P-221
Barsch, Aiko	O-313 P-108 P-154 P-266
Bartolo, François	P-133
Batarseh, Amani	P-7*
Batey, Michael	P-40
Batora, Nick	O-135*
Bauer, Armin	P-74
Bayci, Andrew	P-168
Bearden, Daniel	P-29
Beaumont, Martin	P-176
Beech, Peter	P-129
Beecher, Chris	P-4
Beedgen, Lars	P-201
Beekwilder, Jules	O-71
Beirnaert, Charlie	O-63 P-19*
Beisken, Stephan	O-292
Bekker, Marlize	O-464
bellvert, floriant	P-22*
Ben Jrad, Amani	P-125
Ben-Dor, Shifra	O-106
Beniddir, Mehdi	O-263
Bennett, Mark	P-18*
Bennett, Phillip	O-217 O-244
Berdi, Mikaïl	P-145*
Bergdahl, Ingvar A.	O-289
Berger, Gregor	O-134
Berjanskii, Mark	P-2
Bermingham, Emma	P-247
Bernard, Delphine	P-43
BERTRAND, Cédric	P-125*

Name Last, First	Abstract
* = Presenting Author	
Bhattacharyya, Parthasarathi	P-212
Bhattacharyya Chowdhury, Debjani	P-2
Bienertova-Vasku, Julie	P-203
Bigler, Jeanette	O-179
Binkley, Joe	P-45 P-49
Bird, Virginia	P-225
Bisgaard, Hans	O-347
Bisson, Jonathan	O-263
Bittremieux, Wout	O-301
Bitzer, Michael	P-237
Bjorndahl, Thomas	P-168
Blachier, François	P-176
Blackman, John	O-128
Blankley, Richard	P-64
Blanksby, Stephen	O-462* P-7 P-9
blasco, helene	O-300
Blighe, Kevin	O-315
Blilou, ikram	O-71
Blydt-Hansen, Tom	P-240
bo, Wen	P-36
Boberska, Joanna	P-176
Bocca, Cinzia	O-60 O-300* P-196
Böcker, Sebastian	O-88 O-258
Bodai, Zsolt	O-146*
Boddy, Lynne	O-149
Boerwinkle, Eric	P-221
Bogeva, Elena	O-163
Boldingh, Helen	P-287
Bonneau, Dominique	O-60 P-196
BOONCHAISRI, SIRIWAT	P-126*
Booranasubkajorn, Suksalin	P-83
Borchers, Christoph	P-180
Borewicz, Klaudyna	O-461
Borges, Karin	O-449 P-270*
Borges, Ricardo	O-135 P-94
Borkum, Mark	O-43
Borts, David	P-216*

Name Last, First	Abstract
* = Presenting Author	
Bosch, Dirk	O-71
Boughton, Berin	O-273* P-11
Bousman, Chad	P-277
Boutin, Michel	P-189*
Bowden, John	O-72 P-29
Bowen, Benjamin	P-50
Bowler, Russell	P-250
Brandsma, Joost	O-212*
Bräsen, Christopher	P-51
Bremer, Phil	P-134
Brennan, Lorraine	O-108 O-377*
Britz-Mckibbin, Philip	P-180
Broadbent, James	P-191*
Broadhurst, David	O-179 O-356
Broszczak, Daniel	P-181
Brouwers, Jos	P-289*
Brown, Lindsay	P-279
Bruhn, Laurakay	P-17
Brundiek, Henrike	P-51
Brune, Carly	O-178
Brunius, Carl	O-289 O-309* P-30
Brunk, Elizabeth	O-171
Bryson, Wesley	O-273
Buck, Marion	P-183
Buedenbender, Larissa	O-157*
Buemi, Antoine	P-35
Buenahora, José	P-86
Buggerskov, Katrine	O-237 P-170
Bullen, Hayley	O-184
Bunce, Michael	O-228
Buratovic, Sonja	O-148
Buratti, Martin	P-56
Burgueño, Anna	P-86
Burnham, Matthew	P-286
Butin, Noémie	O-168
Butler, Lisa	O-200
Byard, Roger	O-228
Byer, Jonathan	P-45
Caboni, Pierluigi	O-425
Cabovska, Baiba	P-256

Name Last, First	Abstract
* = Presenting Author	
Cabrera, Diana	P-232*
Cacciatore, Stefano	O-217*
Cai, Zongwei	O-44 O-48 P-121 P-193 P-195 P-206 P-241
Calingacion, Fe	O-156* O-111 O-229
Callahan, Damien	P-129
Cameron-Smith, David	O-82 O-281 P-132
Campos, Christiane	O-477
Cannet, Claire	O-473 P-201*
Caplash, Neena	P-143
Caprioli, Richard	O-273
Caputo, Tiziana	O-62
Carneiro, Sonia	O-282
Carroll, Anthony	O-157
Caruso, Donatella	P-227
Caruso, Massimo	O-179
Casas, Juan	P-221
Casas-Ferreira, Ana	P-269
Cassidy, Ben	O-444
Castelli, Florence	P-145
Castro Gamboa, Ian	P-215*
Castro-Perez, Jose	P-58
Causon, Tim	O-163*
Cavalca, Viviana	P-227
Cave, Nick	P-247
Celedon, Juan	O-315
Cely-Veloza, Willy	P-89
Centelles, Sandra	O-473
Centenera, Margaret	O-200
Cha, Jin Wook	P-75
Chakravarty, Baidy-anath	O-366
Chaleckis, Romanas	O-179 P-64*
Chan, Angelo	P-97 P-104
CHAN, Chi-On	P-71* P-136
CHAN, Shun-Wan	P-71
Chandranipapongse, Weerawadee	P-83

Name Last, First	Abstract
* = Presenting Author	
Chanez, Pascal	O-179
Chang, Chung-ke	P-228*
Chang, Meng-Ting	P-209
Chao de la Barca, Juan Manuel	O-60 P-196
Charidemou, Evelina	O-241
Chatterjee, Niladri	P-151
Chaudhury, Koel	O-366*
Chaudhury, Koel	P-212
Chawes, Bo	O-315 O-347
Cheatham, Carol	P-147
Cheema, Amrita	O-203*
Chekan, Mycola	P-208
Chelliah, Anushka	P-243
Chen, Chen	P-273
Chen, Hsin-Chang	P-222
Chen, Pau-Chung	P-186
Chen, Qiuying	P-14*
CHEN, Sibao	P-71
Chen, Songjie	O-381*
Chen, Wei-Ning	P-144
Chen, Xuyang	P-184*
Chen, Zhongjian	P-193 P-206
Chen, Zhu	O-342
Cheng, Kian-Kai	P-152
Cheng, Ting-Yu	P-216
Chevallier, Olivier	O-69
Chisanga, Malama	O-502
Chisholm, Scott	O-184
Choe, Jeong-Sook	P-282
Choi, Jeong Sook	P-91 P-149
Choi, Phil	P-135*
Choi, Sik-Won	P-167*
Choi, Yongsoo	P-164*
Chong, Eric Tzyy Jiann	P-223
Chong, Jasmine	O-352
Choo, Jocelyn	O-178
Chotewuttakorn, Sirikul	P-83
Choudhury, Priyanka	P-212
Chow, Clement	P-183
Christinal, Rupa	P-205
christison, Terri	P-236
Chung, Bong Chul	P-239
CHUNG, C.K.	P-195

Name Last, First	Abstract
* = Presenting Author	
Chung, Kian	O-179
chunwei, Zeng	P-36
Chupin, Stéphanie	P-196
Cichon, Morgan	P-211*
Claes, Leen	O-63
Clària, Joan	O-168
Clarke, Anthony	P-124
Claude, Emmanuelle	P-34 P-40
Claus, Sandrine	P-176
Clawin-Rädecker, Ingrid	P-77 P-244
Cleland, Gareth	P-256
Clench, Malcolm	P-208
Clinton, Steven	P-211
Clish, Clary	O-279 P-221
Cobbold, Simon	O-302
Cocks, Ben	O-272
Cojocariu, Cristian	P-169
Cole, Laura	P-208
Colnago, Luiz	P-87
Colonna, Maxwell	P-233*
Colsch, Benoit	O-168 O-304 O-319 P-43 P-145
Colson, Kimberly	O-367*
Combs, Martin	P-139
Connolly, Samantha	P-60 P-101*
Cooney, Janine	P-199* P-287
Cooper, Garth	P-16
Cooperstone, Jessica	O-220*
Cooray, Sachindra	P-144*
Copie, Valerie	O-324*
Corcia, Philippe	O-300
Cordiner, Sarah	P-147
Corey, Kathleen	O-279
Correia, Goncalo	O-244
Cort, John	O-43
Costa, Patrícia	O-477
Counihan, Natalie	O-184
Coutinho, Isabel	P-87*
Covaci, Adrian	O-63 P-19
Covington, April	O-146

Name Last, First	Abstract
* = Presenting Author	
Coy-Barrera, Ericsson	P-89*
Coy-Barrera, Ericsson	P-106
Crabb, Brendan	O-184
Creech, Michael	P-17
Creek, Darren	O-184 O-384* P-1 P-70
Crighton, Elly	O-228
Cruickshank-Quinn, Charmion	O-350 P-250*
Currie, Andrew	P-265
Currin-Ross, Denni	P-220*
Cusi, Kenneth	P-191
Cuthberston, Daniel	P-171
Cuttle, Leila	P-181
Cuykx, Matthias	O-63*
D'Aurelio, Marilena	P-14
Dahlén, Sven-Erik	O-179
Dai, Yuqin	P-229*
Damkroeger, Glenn	P-56
Daneshfar, Rambod	P-180
Daniell, James	O-282
Dannhorn, Andreas	O-80*
Datta Ray, Chaitali	O-366
Datwani, Sammy	P-284
Davy, Simon	O-86
Day, Russell	P-107
Dayalan, Saravanan	O-271 P-112
Daygon, Venea Dara	O-111*
de Jong, Felice	P-4
de Koning-Ward, Tania	O-184
de Lauzon-Guillain, Blandine	P-145
De Livera, Alysha	P-1
de Roo, Niels	O-473
De Souza, David	O-271 P-112 P-120
de Souza Pinto Lemgruber, Renato	P-102*
De Tullio, Pascal	P-35
de Vos, Willem	O-83
Dear, Neil	O-178
Déjean, Sébastien	P-133
DeLand, Phillip	P-284* P-286
Deloire, Alain	O-128
Delplancke, Thibaut	O-341
Demetrowitsch, Tobias	P-77 P-244*
Demianova, Zuzana	P-26
Denery, Judith	P-17 P-47*
Denes, Julia	O-425
Depner, Christopher	O-350*
Dervilly-Pinel, Gaud	P-133
Deseo, Myrna	P-139
Desvergne, Beatrice	O-62
Dhariwal, Achal	O-352
Di Giovanni, Nicolas	P-169
Di Minno, Alessandro	P-227
Dias, Daniel	O-86 P-126
Díez-Símoin, Carmen	O-71
Djami-Tchatchou, Arnaud	P-116
Djoumbou Feunang, Yannick	P-3
Djukanović, Ratko	O-179
Djukovic, Danijel	O-249
Do, Kieu Trinh	O-139
D'Occhio, Michael	P-101
Dojahn, Joerg	P-26
Dona, Anthony	P-60 P-101
Dong, Edison	P-180
Dong, Jiyang	P-21* P-152 P-159
Dongliang, Ruan	P-157
Doria, Luisa	P-179 P-188*
Dorrestein, Pieter	O-479
Doyle, Matthew	P-96
Dragsted, Lars	P-24
Dromms, Robert	O-395
Du, Xin	P-148*
Du, Zhi-Yi	P-186
Dubery, Ian	P-114* P-116
Duchoslave, Eva	P-7
Duffy, Sandra	O-157
Dührkop, Kai	O-88 O-258*
Dumont, Laure	O-302
Dunn, Warwick	P-81*
Durainayagam, Brenan	O-82* O-281
Eastwood, Dan	O-149
Eatough, David	P-18
Ebbels, Timothy	O-256
Ebert, Paul	P-97 P-104
Ebrahimi, Parvaneh	O-473
Edison, Arthur	O-135 P-85 P-94 P-233 P-243
Edwards, Patt	P-150
Elahee Doomun, Sheik Nadeem	P-129*
Eliassen, Heather	P-221
Eligini, Sonia	P-227
Elliott, Christopher	O-69
Ellson, Rich	P-284 P-286*
Elsea, Sarah	P-246
Endlich, Karlhans	O-139
Engelsen, Søren	O-473 P-24
Eno, Nathan	P-204
Eriksson, Jan	P-200
Eriksson, Per	O-148
Evans, Anne	O-346 P-96 P-246
Everill, Ian	P-277
Fairchild, Timothy	O-356
Falegan, Oluyemi	O-478*
Fang, Fang	O-473
Fay, Dominik	O-268
Faz, Angel	P-50
Feist, Adam	O-171
Fell, Lorne	P-45 P-49
Fenaille, Francois	O-168* O-319 P-43 P-145
Fenfen, JI	O-44*
Feng, Jia-Hua	P-209
Feng, Jianghua	P-159 P-267*
Fernandes, Joana	P-225
Fernandez, Celine	O-279
Fernandez, Juan Antonio	P-50
Fiehn, Oliver	O-264 P-207
Filipov, Nikolay	P-248*
Fine, Dennis	O-313
Fischer, Christian	O-367
Fischer, David	P-32
Fischer, Steven	P-229
Fisk, Heidi	O-502
Fitzgerald, Melissa	O-89 O-111 O-156 O-229
Fjeldsted, John	O-425
Fleming, Ronan	O-333
Flynn, Albert	O-108
Focant, Jean-François	P-169
Forster, Louise	O-111
Fowler, Stephen	O-179
Fox, Caroline	O-279
Frainay, Clément	O-304
Francis, David	O-220 P-211
Fraser, Karl	O-82 O-281 P-16 P-98 P-132* P-232
Freinkman, Elizaveta	O-346*
Freire, Rafael	P-215
Friedrich, Nele	O-139 O-166 P-175
Friese, Manuel	O-466
Frost, Gary	O-108
Fuchs, Amanda	O-324
Fujimura, Yoshinori	P-252
Fukamachi, Yukihiro	P-38
Fukuda, Shinji	P-190
Fukusaki, Eiichiro	O-303 P-8 P-41* P-46 P-59 P-137 P-260
Fukushima, Atsushi	O-447*
Furuno, Masahiro	P-41 P-46
Gagnon, Marthe	P-120
Gallart-Ayala, Héctor	P-64 O-179
GANDHI, SONIA	P-82*

Name Last, First	Abstract
* = Presenting Author	
Liu, Kwang-Hyeon	P-213 P-230 P-271
Liu, Lian	P-278*
LIU, Liangfeng	O-44
Liu, Pengfei	P-105*
Liu, xili	P-105
Liu, zhiqian	O-272*
Lloyd, Natoiya	O-464 O-500 P-61 P-225* P-285
Loke, Stella	P-129
Lomash, Avinash	P-274*
Long, Michelle	O-279
Long, Sara	O-271* P-112 P-120
Loots, Du Toit	P-93
López, Andrés	P-86
López, Begoña	P-84
Louis, Edouard	P-169
Louw, Roan	P-234
Loza, Matthew	O-179
Lu, Haitao	P-280*
LUAN, Hemi	O-44
Luchinat, Claudio	O-217
Ludwig, Marcus	O-88* O-258
Lutz, Adrian	O-86
Luyf, Angela	P-238
Lycett, Gareth	P-99
Ma, Liyan	P-249
Ma, Yun	P-269
Machitani, Koji	P-46
MacIntryre, David	O-244 O-217
Mackie, Rachel	P-135
Macleod, Ben	P-49*
Macrae, Finlay	P-225
Makela, Johanna	P-132
Maker, Garth	O-228* O-356
Malakar, Dipankar	P-178*
Malan, Leoné	P-234
Malebe, Pelly	O-123
Malek, Nisar	P-237
Malitsky, Sergey	O-106*
Malmodin, Daniel	P-23 P-30
Maltesen, Raluca	O-237* P-170
MAN, Ka-Yi	P-136
Mancano, Giulia	P-176*
Mandal, Rupasri	P-2 P-168 P-180 P-240*
Manega, Chiara	P-227
Manfredi, Giovanni	P-14
Marcellin, Esteban	O-387* P-102 P-283
Marchand, Jérémy	P-133*
Marchesi, Julian	O-474
Marciano, David	O-381
Marczak, Lukasz	P-268* P-208
Mari, Silvia	P-2
Markwald, Rachel	O-350
Marner, Michael	P-74
Marshall, David	O-462
Martin, Damian	O-442
Martineau, Estelle	P-133
Maruvada, Padma	P-182*
Marx, Ute	O-134*
Masson, Perrine	O-256
Masuda, Masashi	P-166
Mathew, Suseela	P-151
Mathon, Caroline	P-64
Matsumoto, Mitsuharu	P-260 P-218
Matthews, Charles	P-221
Mauricio, Rodney	O-135
McCloskey, Douglas	O-171*
McConville, Malcolm	O-271 O-302* P-120
McCouch, Susan	O-111
McDonald, Tanya	O-449*
McGhie, Tony	P-147* P-287
McGorry, Patrick	O-134
McKenzie, James	O-129 O-146 P-179 P-188
McNulty, Breige	O-108
Medin, Jeffrey	P-189
Meeks, Campbell	O-128
Meier, Roland	P-51
Meikle, Peter	O-436
Meinke, Marie	O-134
Meitei, Ningombam	P-58
Meitei, Sanjib	P-9
Melander, Olle	O-279
Mellett, Natalie	O-436
Melnik, Alexey	O-479
Menni, Cristina	O-139 P-221
Menon, Smrithi	O-203
Meruva, Naren	P-256
Mervant, Loic	P-43*
Methling, Karen	P-272*
Metz, Thomas	O-43 O-104 O-245*
Meuwis, Marie-Alice	P-169
Meyer, Klaus	P-55
Meyer, Sven	O-313 P-55* P-266
Meysman, Pieter	O-301 P-19
Michielsen, Charlotte	O-108
Miki, Akiko	P-252
Milan, Amber	O-82 O-281* P-150
Miléa, Dan	P-196
Millard, Pierre	P-22
Miller, J.	O-190
Miller, Luke	O-346 P-96 P-246*
Miller, Marcus	P-246
Miranda, Victor	O-82
Mistrik, Robert	P-88
Mitchell, Cameron	O-82 O-281
Mitchell, Cameron	P-150
Mitchell, Sarah	O-281 P-150
Mitchell, Todd	P-7
Miura, Daisuke	P-252*
Miyagawa, Hiromi	P-28 P-90
Miyagi, Atsuko	P-109*
Miyaura, Kazutoshi	P-153
Moazzami, Ali	P-200
Moffat, Caroline	P-288
MOK, Daniel Kam-Wah	P-71 P-136*
Molendijk, Jeffrey	O-154*
Molina, Jennifer	P-171
Moon, Kwang Hyun	P-162
Moonen, Glenn	O-387 P-283
Moore, Steven	P-221
Morales, Rodrigo	P-70
MORE, TUSHAR	P-231*
Moreau, Richard	O-168
Moreno, Pablo	O-292 O-414*
Mori, Mihoko	P-174
MORILLON, Aude-Claire	P-251*
Morlacchi, Pietro	P-171*
Morningstar, Jordan	O-279
Mose, Richard	O-123
Moseley, Hunter	O-77
Moss, Jonathan	P-266*
Motamedchaboki, Khatereh	P-10
Mote, Ryan	P-248
Motoike, Ikuko	O-230 P-100 P-262*
Mourad, Michel	P-35
Moutafis, George	O-387 P-283
Moyna, Guillermo	P-86
Mroz, Anna	O-80 O-129 P-179* P-188
Mrzic, Aida	O-301*
Mueller, Jochen	P-135
Muhamadali, Howbeer	O-502
Müller, Carsten	O-149
Müllner, Elisabeth	P-200
Murgia, Antonio	O-425
Musgrave, Ian	O-228
Mykkänen, Hannu	O-461
Nakabayashi, Ryo	O-407*
Nakagawa-Goto, Kyoko	P-209
Nakamura, Hirofumi	P-111
Nakamura, Sadao	P-39

Name Last, First	Abstract
* = Presenting Author	
Nakano, Yosuke	P-8*
Nakayama, Yasumune	P-59*
Nash, William	P-81
Natera, Siria	P-119
Nauck, Matthias	O-166 P-175
Naz, Shama	O-179 P-64
Nepomuceno, Alexandre	P-87
Neubauer, Cajetan	P-27
Neumann, Steffen	O-292 O-414
Neuweger, Heiko	P-266
Nicholson, Jeremy	O-222 O-239 O-256 O-474
Nicolotti, Luca	O-500 P-61* P-285
Niehs, Sarah	P-272
Nielsen, Jens	O-282
Nielsen, Lars	O-387 P-102 P-283
Nieto, Sofia	P-204*
Nikou, Theodora	P-154
Nishi, Akinori	P-218
Nishizawa, Tomoko	O-447
Nordlund, Emilia	O-461
Northen, Trent	P-50
Nothaft, Harald	P-85
Nothias, Louis-Felix	O-479*
Nothias-Scaglia, Louis-Felix	O-88
Novotny, Janet	O-220
Nozaki, Tomoyoshi	P-174 P-210
Ntai, Ioanna	P-66*
Nugent, Anne	O-108
Nunez, Jamie	O-43* O-104
Nurkanto, Arif	P-210*
Nyarukowa, Christopher	O-123
Nye, Leanne	P-18
O'Donovan, Claire	O-445
Obkircher, Markus	P-51
O'Brien, Allyson	O-271 P-112

Name Last, First	Abstract
* = Presenting Author	
O'Brien, Jake	P-135
O'Brien, Terence	P-270
O'Donovan, Claire	O-414
Ogiwara, Atsushi	P-37
Ogiwara, Shohei	P-254
Oh, Sehyun	P-281*
Ohbuchi, Katsuya	P-218*
Ohk, Boram	P-163
Ohno, Satoshi	O-328
Okamoto, Yoshinori	P-254*
Okun, Jürgen G.	P-201
O'Leary, Jade	O-149*
Oliver, Richard	P-288
Olshansky, Gavriel	P-1*
Olson, Loren	P-44*
Omori, Kazuo	P-41
Ono, Hiroshi	P-111
Oppermann, Madalina	P-56
Orellana, Camila	O-387 P-283
Oresic, Matej	P-221
Orford, Elise	O-241
Organtini, Kari	P-256
Ortega, Alejandra	O-57*
Osaki, Shusuke	P-46
Östman, Johnny	P-200*
O'Sullivan, John	O-279*
Outchkourov, Nikolay	O-71
Ovenden, Ben	O-111
Ovenden, Simon	P-277*
Overduin, Michael	P-180
Paananen, Jussi	O-461
Paine, Mark	P-99
Paley, Suzanne	O-66*
Palmer, Andrew	O-268 O-273
Palo, Titchaporn	P-83
Palsson, Bernhard	O-171
Panagos, Charalampos	O-135
Panchagnula, Dr. Venkateswarlu	P-205
Panchal, Sunil	P-279
Pandis, Ioannis	O-179
Pankratov, Leonid	O-169
Panse, Christian	P-32

Name Last, First	Abstract
* = Presenting Author	
Pantelis, Christos	O-134 P-277
Papan, Cyrus	P-26
Parachin, Nádia	O-477
Parat, MO	O-89
Park, Hee-Sook	P-73
Park, Jin Hwan	P-103
Park, Ki Do	P-167
Park, Soo-Yun	P-128
Park, Sujin	P-103
Park, Yoo Kyoung	P-163
Park, Yu Jin	P-160*
Parker, Tony	P-181
Parmar, Dharmeshkumar	P-205*
Parra-Llorca, Anna	O-169
Pascual, Jose Antonio	P-50
Pathan, Nazima	P-177
PATIL, ANUJA	O-89*
Patil, Chandrashekhar	P-125
PATIN, FRANCK	O-300
Patsiouras, Heather	P-40*
Pavagadhi, Shruti	P-33*
Pavlova, Tereza	P-203
Peake, David	P-172 P-27* P-38* P-56 P-236
Pearce, Jake	O-239
Pedersen, Anders	P-23* P-30
Peiffer, Jeremiah	O-356
Pekar Second, Tonya	P-27
Pekkinen, Jenna	O-461
Peng, Yang	P-198
Perdones-Montero, Alvaro	O-244
Pereira, Alexandre	P-221
Pereira Das Neves, Virginia	P-86
Perera, Ranjan	P-53
Pessia, Alberto	O-83
Petersen, John	O-197
Peterson, Jeff	P-48*
Peterson, Randall	O-279
Petrusheva, Dragana	O-163
Pettigrove, Vincent	P-120

Name Last, First	Abstract
* = Presenting Author	
Pflanz, Ralf	P-110
Pham, Tuan Hai	P-56 P-235
Phapale, Prasad	O-268*
Phelps, David	P-188
Piater, Lizelle	P-114 P-116
Pichler, Bernd	P-237
Pieters, Luc	P-19
Pietrowska, Monika	P-208 P-268
Pietzner, Maik	O-139 O-166* P-175*
Pillai, Manoj	P-151 P-178
Pilon, Alan	P-215
Pinu, Farhana	O-282
Pitteri, Sharon	O-381
Place, Benjamin	P-29
Pless, Ole	O-466
Plise, Emile	P-44
Plummer, Kim	P-130
Poad, Berwyck	O-462
Polipalli, Sunil	P-274
Pomfret, Aislinn	P-216
Poppitt, Sally	P-16
Porat, Ziv	O-106
Porro, Benedetta	P-227
Portais, Jean-Charles	P-22
Poso, Antti	O-461
Postle, Tony	O-212
Poutanen, Kaisa	O-461
Powell, Katie	P-60
Powell, Kevin	P-130
Power, John	O-387 P-283
Prabhala, Bala	O-200
Prakash, Sangeeta	O-111
Prashar, Suvira	P-161
Pras-Raves, Mia	P-238
Priebbenow, Daniel	P-70
Procaccio, Vincent	O-60 P-196
Prodhan, Utpal	O-281
Proffitt, Tina	O-134
Proitsi, Petroula	P-269
Protsyuk, Ivan	O-268 O-479

Name Last, First	Abstract
* = Presenting Author	
Seifert, Stephan	P-77 P-244
SEKIYAMA, Yasuyo	P-111*
Selegato, Denise	P-215
Sen, Arundhuti	P-269
Seo, Woo Duck	P-167 P-276*
Seong, Sook Jin	P-163 P-264
Serino, Takeshi	P-28 P-90
Serra-Cayuela, Arnau	P-2 P-180
SEUBNOOCH, PATCHARAMON	P-83*
Seyfried, Florian	O-474
Seymour, Jamie	P-184
Shah, Dimple	P-256
Shah, Svati	P-221
SHAM, Tung-Ting	P-71
Sharma, Pankaja	O-281 P-150
Shaw, Dominick	O-179
Shaw, PN	O-89
Sheen, David	P-29
Shen, Chen-Yang	P-228
Shen, Guiping	P-152 P-159*
Shen, Lu	P-51
Shen, Xiaotao	P-78*
Shen, Yuh-Chiang	P-242
Sherman, Emma	O-442*
Shi, Lin	O-289* O-309
Shiga, Kazuki	P-137
Shimma, Shuichi	P-6*
Shimobori, Chika	P-218
Shimomura, Iichiro	P-41
Shin, Eunju	P-162
Shin, Jeong-Sook	P-164
Shin, Min-Jung	P-282
shindo, Mitsuru	P-252
Shiomi, Kazuro	P-174
Shiota, Teruhisa	P-28 P-90
Shirota, Matsuyuki	O-230 P-262
Shkodra, Maltina	P-269
Shon, Jong Cheol	P-213* P-230

Name Last, First	Abstract
* = Presenting Author	
Shrestha, Aahana	P-150*
Shu, Xiao-ou	P-221
Shuichi, Shimma	P-33
Shyur, Lie-Fen	P-209*
Siddiqui, Ghizal	O-384 P-70
Siebers, Bettina	P-51
Silcock, Patrick	P-134
Silcock, Paul	P-169
SIM, INSEON	P-162*
Sim, Joon-Soo	P-123
Simard, Gilles	O-300 O-60 P-196
Simón-Manso, Yamil	P-29
Simpson, Julie	P-1
SIMSEK, ERHAN	P-259*
Sinclair, Georgia	O-271 P-112*
Sinclair, Ian	P-284 P-286
Singh, Akanksha	P-151* P-178
Singh, Digar	P-263
Singh, Sukhvinder	P-143
Siow, Yaw	P-161
siqi, Liu	P-36
Sitapara, Nishit	P-44
Sjödin, Anders	O-281
Skaaby, Tea	O-166
Skipp, Paul	O-179
Sleeman, Jonathan	P-40
Smallfield, Bruce	P-140
Smallwood, Heather	P-68
Smidt, Hauke	O-461
Smilde, Age	O-473
Smith, David	P-150
Smith, Paul	O-464
Smyth, Heather	O-229
Snel, Marten	O-178* O-200
Snell, Peter	O-111
Snyder, Michael	O-381
So, Pui-Kin	P-138*
Soares, Renata	O-80
Soares, Renata	P-179

Name Last, First	Abstract
* = Presenting Author	
Soga, Tomoyoshi	O-328 P-5 P-6 P-31 P-166 P-174
Sokkalingam, Nandhini	P-48
Solberg, Rønnaug	O-169
Soma, Yuki	P-28 P-90
Somerset, Amy	P-168*
Son, Su	P-67*
Song, Jaeeun	P-123
Song, Jiangning	O-458
Sørensen, Klavs	P-24
Souza, Amanda	P-66 P-169*
Souza, Ana	O-179
Spacil, Zdenek	P-203*
Spector, Tim	O-139
Spjuth, Ola	O-414
Spotbeen, Xander	O-200
Spraggins, Jeffrey	O-273
Spratt, Heidi	O-197*
Spraul, Manfred	O-473 P-201
Srinivas, Pothur	P-221
Srinivasan, Parthi	P-269
Srivastava, Anubhav	O-184* O-384 P-70
Stanstrup, Jan	O-147*
Stead, Sara	P-131*
Steenackers, Hans	O-167
Steenkamp, Paul	P-114 P-116
Steinbeck, Christoph	O-292 O-414 O-445
Sterk, Peter	O-179
Stevens, Victoria	P-221
STEVENSON, TREVOR	P-126
Stewart, Joseph	P-107
Steyn, Adrie	P-204
Stockley, Creina	P-225
Stoessel, Daniel	O-466*
Stoll, Thomas	O-154
Stolzenger-Solomon, Rachael	P-221

Name Last, First	Abstract
* = Presenting Author	
Stratton, Kelly	O-245
Stratton, Tim	P-66 P-88*
Strittmatter, Nicole	O-80
Styczynski, Mark	O-395*
Subaihi, Ali	O-502
Subbaraj, Arvind	P-98*
Subramani, Elavarasan	O-366 P-212
Sugimoto, Masahiro	P-226*
Sugitate, Kuniyo	P-28 P-39* P-90
Suh, Dong Ho	P-162 P-263*
Suklje, Katja	O-128
Sulek, Karolina	P-187*
Sumarah, Mark	O-190*
Sumner, Lloyd	O-313*
Sun, Lili	P-249*
Sun, Qin	P-246
Sundekilde, Ulrik	P-24
Suryawanshi, Rahul	P-125
Suzuki, Kenichi	P-28 P-90
Swales, John	O-80
Swarup, Sanjay	O-388 P-33 P-113
Swinnen, Johan	O-200
Szubin, Richard	O-171
Szymanski, Christine	P-85
Szymczak, Silke	P-77 P-244
Tacke, Ulrich	P-261
Tadaka, Shu	P-262
Takahashi, Masatomo	P-28 P-90
Takats, Zoltan	O-80 O-129 O-146 O-222 O-244 P-179 P-188
TAKEBAYASHI, TORU	P-31
Takeo, Emi	P-6
Taketani, Yutaka	P-166
Takeuchi, Makoto	O-93
Tamura, Yoshio	P-153*
Tan, Gina	P-68*

Name Last, First	Abstract
* = Presenting Author	
Tanaka, Kazuki	P-190*
Tang, Huiru	O-127* P-219
Tang, Zhi	P-121
Tansawat, Rossarin	P-207*
Tantisira, Mayuree	P-207
Tappel, Ryan	P-102
Tatano, Hiroshi	P-166*
Tautenhahn, Ralf	P-66 P-236
Tavi, Pasi	O-461
Taylor, Carla	P-161
Team, MetaboLights	O-445
Team, PhenoMeNal	O-445
Teeguarden, Justin	O-104
Teerapattarakan, Narudol	P-207
Tellström, Verena	P-76
Temprosa, Marinella	P-42* P-221
Tena, Maria	P-269
Tenderholt, Adam	P-48
Tenori, Leonardo	O-217
Thabut, Dominique	O-304
THABUT, Dominique	O-319
Thamsermsang, Onusa	P-83
Thayyil, Sudhin	P-202
Kuligowski, Julia	P-202
Thiele, Ines	O-333
Thomas, Aurelien	O-62
Thomas, Dennis	O-43 O-104
Thomas, Kevin	P-135
Thomaz, Fernanda	P-279*
Thompson, Chris	P-72* P-154
Thornton, Ruth	P-265
Thuesen, Betina	O-166
Thurston, Sarah	P-177
Tilley, Leann	O-302
Tillner, Jocelyn	O-222
Timm, Wiebke	P-154*
Toal, Doug	P-246
Tokida, Takeshi	P-111
Tolson, Rose	O-239

Name Last, First	Abstract
* = Presenting Author	
Tomita, Masaru	P-5 P-31 P-166 P-190
Toojinda, Theerayut	O-69
Torta, Federico	O-154
Touboul, David	O-479
Towers, Mark	P-18 P-34 P-40
Tran, Carol	P-47
Treebak, Jonas	P-187
Trefz, Friedrich K.	P-201
Tremoli, Elena	P-227
Trengove, Robert	O-228 O-356 P-265
Trim, Paul	O-178 O-200*
Trower, Tania	P-199
Trygg, Johan	O-517*
Tsalenko, Anya	P-17
Tscharke, Ben	P-135
Tseng, Wei-Chen	P-186*
Tsugawa, Hiroshi	O-264*
Tugizimana, Fidele	P-114
Tugizimana, Fidele	P-116*
Tull, Dedreia	O-271 P-112 P-120*
Tuomainen, Tomi	O-461
Turkoglu, Onur	P-168
Turner, Zachary	P-248
Turnu, Linda	P-227*
Tybring, Gunnel	P-30
Tzoulakim, Joanna	P-221
Uawisetwathana, Umaporn	O-69*
Ubhi, Baljit	P-4 P-10* P-26* P-44 P-53 P-151 P-178 P-191
Ueda, Koji	P-254
Ulmer, Candice	O-72* P-29
Ulrich, Cornelia	P-221
Umashankar, Shivshankar	O-388* P-113

Name Last, First	Abstract
* = Presenting Author	
Uppal, Karan	P-248
Urban Alandete, Lourdes	P-146*
Usui, Yasuhiro	P-111
Uusitupa, Matti	O-461
Valgepea, Kaspar	P-102
Van de Bittner, Kyle	O-278*
van der Westhuizen, Francois	P-234
van Deventer, Cynthia	P-234
van Dijk, Aalt-Jan	O-71
van Duynhoven, John	O-473
van Kampen, Antoine	P-238
van Klink, John	P-140
Van Poucke, Christof	P-131
van Weeghel, Michel	P-238*
Vanavichit, Apichart	O-69
Vanhaecke, Lynne	P-131
Vanhaecke, Tamara	O-63
Vardi, Assaf	O-106
Vargas Medina, Sandra	P-106*
Vasan, Ramachandran	O-279
Vassiliadis, Simone	P-130
Vaz, Frédéric	P-238
Vazquez-Fresno, Rosa	P-2 P-180
Velagapudi, Vidya	O-83*
Velkov, Tony	O-458
Venables, Michelle	O-241
Venter, Leonie	P-93
Venter, Marianne	P-234*
Vento, Máximo	O-169
Veras, Henrique	O-477
Verdegem, Dries	O-77*
Verhoeven, Koen	P-65
Vermaelen, Wesley	O-77
Verplanken, Kaat	P-131
Vervaart, Martin	P-238
Veselkov, Kirill	P-40
Veyrat-Durebex, Charlotte	O-300 P-196
Viant, Mark	P-81
Viberg, Henrik	O-148
Vidova, Veronika	P-203

Name Last, First	Abstract
* = Presenting Author	
Villas-Boas, Silas	P-141 O-442
Virkud, Yamini	O-314* O-315 O-347
Vissers, Johannes	P-57 P-58
Vitale, Marilena	O-461
Vogel, Hans	O-478
Voolstra, Christian	O-57
Vorkas, Panagiotis	O-256
Vosloo, Andre	P-93
VOURCH, PATRICK	O-300
Vozza, Nicolas	P-2
Wada, Koji	O-93
Wagner, Karl	P-150 O-281
Walejko, Jacquelyn	P-243*
Walker, Douglas	P-248
Walther, Bernard	O-256
Walton, Janette	O-108
Wanders, Ronald	P-238
Wang, Dezhen	O-150 P-127*
Wang, Guangwen	O-381
Wang, Jin	O-127
Wang, Lei	O-401*
Wang, Ming	P-108
Wang, Ning	O-93
Wang, Thomas	O-279
Wang, Xijun	O-511*
Wang, Yao	O-150 P-127
Wang, Yulan	O-127 P-219*
Wanichthanarak, Kwanjeera	P-83
Ward, Leigh	P-279
Wariishi, Hiroyuki	P-252
Washton, Nancy	O-43
Wassell, Sara	O-241
Waterham, Hans	P-238
Waters, Katrina	O-245
Webb-Robertson, Bobbie-Jo	O-245
Weber, Ralf	P-81
WEI, Juntong	P-195
WEI, Juntong	P-241*
Weir, Jacquelyn	O-436
Weiss, Greta	O-184

Name Last, First	Abstract
* = Presenting Author	
Weiss, Nicolas	O-304 O-319
Weiss, Scott	O-314 O-315 O-347
Wellard, Mark	P-181 O-134
Wells, Martin	P-251
Wen, He	P-75
Wen, Shi	P-267
Wenk, Markus	O-154
Wenk, Patricia	P-237*
Werner, Erwan	O-256
Wesselingh, Steve	O-178
Westley, Chloe	O-502
Wheelock, Craig	O-179 P-64
Wherritt, Daniel	O-313
White, Debbie	P-177
White, Emma	O-146
Widlak, Piotr	P-208 P-268
Wiggs, Bobby	P-246
Wijeyesekera, Anisha	P-177*
Wijffels, Gene	P-220
Wilkinson-White, Lorna	P-60* P-101
Williams, Jonathan	P-18 P-57
Williams, Nari	P-118
Wilschut, Rutger	P-65
Wilson, Steve	P-256*
Wimmer, Reinhard	O-237 P-170
Wingfield, Jon	P-284 P-286
Wishart, David	P-2* P-3 P-15 P-168 P-180 P-240
Witt, Matthias	P-72 P-154
Wohlgemuth, Gert	O-264 P-207
Wohlgemuth, Roland	P-51
Wojakowska, Anna	P-208* P-268
Wolber, Frances	P-232
Wolfer, Arnaud	O-256
Wong, Chi Chun	O-48
WONG, Chi-Ming	P-195
WONG, Ka-Hing	P-136
Wood, Stephen	O-134
Work, Thierry	P-107
Worrall, Simon	P-279
Wright, Kenneth	O-350
Wszolek, Agata	P-51
Wu, Lilan	O-48
Wu, Qianxin	O-474
Wu, Vincen	O-222*
Wu, Zhanxuan	O-474 P-16*
Wu, Zhexue	P-271
Wulff, Jacob	P-246
Wunderlich, Dirk	P-76
Wurster, Martina	P-272
Xia, Ashley	P-182
XIA, JIANGUO	O-352*
Xia, Yinyin	P-184
Xiang, Li	P-241
Xiang, Wenying	P-245
Xie, Yumin	P-245
Xu, Jin	P-269*
Xu, Jingjing	P-152* P-159
Xu, Meimei	O-172
Xu, Sibei	O-171
Xu, Xiangnan	P-21
Xu, Yun	O-69
Y.L.Li, Adelicia	P-33
Yadori, Hiromi	P-41
Yakkundi, Shirish	P-251
Yamada, Kayoko	P-28 P-90
Yamamoto, Masa-hiro	P-218 O-230 P-262
Yamamoto, Yuichi	P-41
Yamanaka-Okumura, hisami	P-166
Yamashita, Toshiyuki	P-28 P-90
Yan, Jiacheng	P-121
Yan, Jin	O-150 P-127
Yang, Chunxue	P-195* P-241
Yang, Dong Sik	P-103*
Yang, Qiong	O-279
Yang, Wu	P-245
Yang, Yueze	P-94
Yang, Zhiyi	P-193 P-206*
Yanhong, Gao	P-122*
Yaniguchi, Moyu	P-8
Yanjun, Hong	P-157
Yasuda, Jun	O-230
Yatapan, Supavadee	O-69
Yaxley, John	P-183
Yeh, Christine	O-381
Yeo, Hock Chuan	P-95*
Yesiltepe, Yasemin	O-43 O-104
Yi, Hannah	P-282
Yi, Xiaojiao	P-193 P-206
Yin, Xiaofei	O-377
Yin, Xuejiao	P-214*
Yokoi, Yasuto	P-38
Yong, Yoong Soon	P-222*
Yonghai, Lu	P-122
Yoon, Young-Ran	P-163 P-264
Yost, Richard	O-453*
Young, Reuben	O-462
Yousefzadeh, Laleh	P-25
Yssel, Anna	O-167*
Yu, Dingyi	P-119
Yugi, Katsuyuki	O-328
Yun, Jeong Hyun	P-142
Yusianto, Yusianto	O-303
Yuzuki, Masanobu	P-137
Zaher, Sara	P-177
Zanetti, Krista	P-221
Zang, Tuo	P-181*
Zaragoza, Nicolas	O-387 P-283*
Zarate, Erica	P-63*
Zareian, Mohsen	P-134*
Zeng, Nina	O-281 P-150
Zhan, Bohan	P-267
Zhang, Dabao	P-273
Zhang, Guanshi	P-240
Zhang, Hongwu	P-249
Zhang, Hua	O-341 O-342 P-184
Zhang, Jiamin	P-180
Zhang, Min	P-273*
Zhang, Yinwen	P-94
Zhang, Yiran	P-2 P-180
Zhang, Ziamin	P-2
Zhanlong, Mei	P-36*
Zhao, Chao	P-121*
Zhao, Hua	P-221
Zhao, Jinxin	O-458
Zhao, Shaying	P-233
Zheng, Baohui	O-279
Zheng, Hui	O-279
Zheng, Laura	P-250
Zheng, Shuning	O-106
Zheng, Xueyun	O-104
Zhou, Lihong	O-127
Zhou, Xiaobo	O-342*
Zhou, Zhiwei	O-172* P-69
Zhu, Wentao	O-150* P-127
Zhu, Xiaoyang	P-219
Zhu, Yan	O-458*
Zhu, Zhengjiang	O-172 P-69* P-78
Zierer, Jonas	O-139*
Zijlstra, Andries	O-478
Zimmerman, Jeffery	P-216
Zink, Erika	O-245
Ziv, Carmit	O-106
Zongwei, Cai	P-157
Zou, Zhongmei	P-249
Zubeil, Florian	P-74*
Zwicker, Paula	P-272

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METABOLOMICS 2017

13th Annual Conference of the Metabolomics Society

BRISBANE, AUSTRALIA June 25—29

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