



## Metabolomics 2016 Poster Abstracts

### Poster #: 1

**Abstract #:** 2083

**Abstract Title:** MoOD – Our novel Metabolomics Outlier Detector for R-Shiny

**Authors:** Alexander Cecil, Cornelia Prehn, Jerzy Adamski,

**Presenting Author Affiliation:** Helmholtz Center Munich

### Abstract Submission:

Targeted or non-targeted metabolomic analyses are, as all in vitro or in vivo measurements, prone to technical errors. These errors can occur at several states of sample preparation and may lead to, in worst cases, nonsensical data which can't be rescued even by the most sophisticated biostatistical algorithms. In less severe cases these technical errors are, if not detected early on, responsible for lengthy and more extensive data analysis.

To minimize this risk we created the software package “MoOD” for R Shiny. The aim of the software is to provide for non-expert users an easy to use interface to quickly check the quality of sample preparation and measurement.

The user is presented with an intuitive GUI and starts with the upload of a text file. MoOD has been developed initially for data derived from MetIDQ® containing metabolomic data of the Biocrates® p150 and p180 assays. The calculations are primarily presented in two tabs of the output pane: 1) general and 2) detailed information of data composition of marker metabolites. The marker metabolites are representative of the full metabolite spectrum of the two assays and grouped by their chemical properties.

The general information pane provides the user with QQ-plots of the metabolites combined with outlier detection by boxplots of log2 transformed and original scale data. From this pane it is directly visible if outliers in the data have been detected. The detailed information tab provides the user with detailed outlier detection for each marker metabolite.

For now, MoOD is restricted to targeted metabolomic measurements with p150 and p180 kits. However, the code is very adaptable and other types of targeted or even non-targeted metabolomics – as well as more biostatistics for the whole dataset – are going to be included in future versions of the software.

**Poster #: 2****Abstract #: 2183****Abstract Title:** AddClique: A network-based algorithm for the identification of adducts in LC/MS metabolomics**Authors:** Oriol Senan Campos, Antoni Aguilar-Mogas, Miriam Navarro, Oscar Yanes, Marta Sales-Pardo, Roger Guimerà,**Presenting Author Affiliation:** Universitat Rovira i Virgili**Abstract Submission:**

LC/MS is the most common experimental setup for untargeted metabolomics. In a typical LC/MS experiment, compounds are first separated in the chromatography and then ionized at the spectrometer, where they are analyzed. In this process, molecules from the same metabolite can undergo different transformations, usually incorporating or losing precise molecular moieties. Each of these newly formed molecules or adducts of the same metabolite produces a different signal in the spectrometer, increasing the complexity of the analysis. Correct interpretation of these signals is crucial for a rigorous and accurate metabolomics experiment. To aid in this interpretation we have developed AddClique, a network based algorithm that is able to systematically discriminate adducts from the same metabolite from those from another metabolite, and then identify them.

AddClique first computes correlations between peaks of the chromatogram to obtain the probability that those peaks correspond to adducts of the same molecule. With these probabilities AddClique builds a network, with adducts as nodes and probabilities as links. Then, AddClique identifies groups of fully connected components (cliques), as these are the most probable adducts of the same metabolite. Finally AddClique identifies the adducts with their masses as well as the metabolite.

We tested our algorithm with datasets of increasing complexity: from single molecule experiments to a complex biological sample. We show that AddClique is a valid tool for the identification of adducts and can be incorporated to the regular workflow in the analysis of metabolomics data.

**Poster #: 4**

**Abstract #: 2359**

**Abstract Title:** Quantification of bioactive N-acylethanolamines and in human plasma

**Authors:** TAKAMASA ISHIKAWA, AKIYOSHI HIRAYAMA, Toru Takebayashi, Tomoyoshi Soga, Masaru Tomita,

**Presenting Author Affiliation:** Institute for Advanced Biosciences Keio University

**Abstract Submission:**

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, however, human plasma metabolite database named NIST Standard Reference Material for Human Plasma (SRM 1950) does not include this metabolite. 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-acylethanolamine metabolites.

**Poster #: 5****Abstract #: 2411****Abstract Title:** Metabolic changes in prefrontal cortex of humans, chimpanzees and macaques during postnatal development.**Authors:** Ilia Kurochkin, Philipp Khaitovich,**Presenting Author Affiliation:** Skolkovo Institute of Science and Technology**Abstract Submission:**

Human evolution is characterized by changes in brain size and organization. It has long been suggested that these structural changes are linked to modifications in brain metabolism. In our study we assessed metabolic features unique to human brain by measuring intensity of 5750 metabolic peaks detected using liquid chromatography coupled with mass spectrometry in positive ([+] LC-MS) and negative ([-] LC-MS) ionization mode in a specific area of dorsolateral prefrontal cortex of 40 humans, 40 chimpanzees, and 40 rhesus monkeys. In all species samples span the entire development: from 2 days to 61 years in humans, from newborn to 42 years in chimpanzees, and from 13.6 weeks post-conception to 21 years in macaques. In order to assess metabolite concentration changes after death, we further conducted measurements in additional postmortem samples of two rhesus macaques. We show that the brain metabolome undergoes substantial changes with approximately 75% of detected metabolites showing significant concentration changes with age. Notably, 80% of these metabolic changes differ significantly among species with approximately two-fold greater number of changes taking place on the human evolutionary lineage compared to the chimpanzee lineage. The excess of human-specific divergence was not distributed uniformly across lifespan, but peaked between 40 and 55 years of human age. Coupling of human-specific metabolic changes with corresponding changes in mRNA abundance signed out TCA cycle and arginine and proline metabolism, as well as number of other pathways, as the ones enriched in human-specific changes.

**Poster #: 6**

**Abstract #: 2428**

**Abstract Title:** Assessing the penetration of antimicrobials into *Pseudomonas aeruginosa* biofilms using ToF-SIMS

**Authors:** Sarah Davies, Sarah Fearn, David McPhail, Jacob Bundy,

**Presenting Author Affiliation:** Imperial College London

**Abstract Submission:**

Cells that exist as part of a biofilm are much less susceptible to antimicrobials than are planktonic cells. Biofilms commonly grow on medical equipment and inserts such as catheters, and are often extremely difficult to remove. *Pseudomonas aeruginosa* biofilms grow in the lungs of cystic fibrosis patients, causing chronic infections. We have used Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) to compare the penetration of three clinically relevant antimicrobials (tobramycin, ciprofloxacin and gallium) into *P. aeruginosa* biofilms. ToF-SIMS combines high spatial resolution (up to 300 nm) with high sensitivity.

We grew *P. aeruginosa* biofilms on indium tin oxide (ITO)-coated glass, as a flat, electrically conductive substrate is preferred for ToF-SIMS analysis. We confirmed biofilm formation on this substrate with SEM. ITO coated glass pieces were incubated in liquid *P. aeruginosa* culture (PA14 retS) for 96 hours to allow biofilm formation, then removed before the addition of a mixture of antimicrobials with a range of exposure times. Samples were lyophilized before MSI analysis.

MS imaging was carried out at a submicron resolution (with a 0-800 m/z range) using a TOF.SIMS 5 secondary ion mass spectrometer (IONTOF). This instrument can operate in two modes. The first is a static mode for surface MS imaging (X-Y axis) using a Bi<sup>3+</sup> ion beam, causing minimal damage to the sample surface. The second is a dynamic, destructive mode for depth profiling, using an argon cluster primary ion beam to sputter material from the sample, enabling the acquisition of sequential MS data on the Z-axis.

By analysing pure antibiotic standards, and depth profiling through the biofilm to the substrate, we were able to obtain and compare profiles of antimicrobial penetration into the biofilm, as well as identifying endogenous chemical gradients within the biofilm itself.

**Poster #: 7**

**Abstract #: 2569**

**Abstract Title:** Chemometric bioinformatics - developments and application in health and disease

**Authors:** Henrik Antti, Pär Jonsson,

**Presenting Author Affiliation:** Umeå university

**Abstract Submission:**

Chemometric bioinformatics is a concept where chemometric methods, i.e. design of experiments (DOE) and multivariate analysis by means of projections (MVA), are applied in solving, and developing new tools for, bioinformatic problems. Our ambition is to increase the information output from metabolomics data by further developments and refinements of chemometric methods and strategies. In this presentation the use of DOE and MVA methods in metabolomics studies will be critically discussed and suggestions of novel approaches utilizing the uniqueness of the chemometric methodology will be given. Special emphasis will be on how to increase the sensitivity of biomarker or biomarker pattern detection as well as how to obtain a more comprehensive and correct interpretation of the systematic metabolic pattern changes and how to deal with this statistically. Examples from studies including matched or dependent samples will be given and discussed. In addition, the impact and use of orthogonal systematic variation in metabolomics data will be given specific attention for defining the significance of metabolites or patterns thereof. All presented strategies will be exemplified in real clinical studies ranging from early disease detection to treatment response and controlled interventions.

**Poster #: 8**

**Abstract #: 2027**

**Abstract Title:** Integrated gene expression and metabolomics analysis defines molecular cancer signatures

**Authors:** Ewy Mathé, Elizabeth Baskin, Senyang Hu,

**Presenting Author Affiliation:** The Ohio State University

**Abstract Submission:**

Cancer remains a leading cause of death. Every year, over 14 million people are diagnosed with cancer worldwide and over 8 million people will die of the disease. With the strong potential of using metabolites as guides for predicting early diagnosis, prognosis, and treatment outcome, metabolomics has gained momentum in the recent years. Oftentimes though, the interpretation of metabolomics profiles, especially in large untargeted studies, is challenging and the biological mechanisms underlying cancer-specific profiles are frequently unknown. Yet understanding the regulation of metabolic enzymes involved in producing cancer phenotypes is critical and could facilitate the search for novel therapeutic targets. To this end, a global approach that integrates gene expression with metabolite measurements is proposed. Importantly, this approach could be extended to the integration of other omics data (e.g. proteomics, epigenomics).

Highly correlated gene:metabolite pairs may reveal genes that directly or indirectly affect the abundances of metabolites related to a specific cancer phenotype. We hypothesize that gene:metabolite correlations are associated with different phenotypes and that these pairs may reveal key biological mechanisms that affect different phenotypes. Leveraging the public NCI-60 cell line data, we applied a linear model,  $m = g + c + g:c$ , where  $m$  are metabolite abundances,  $g$  are gene expression values,  $c$  is cancer type (e.g. leukemia, prostate), and  $g:c$  is the interaction between gene expression and cancer type. A statistically significant interaction p-value indicates that the corresponding gene:metabolite pair is highly correlated in one cancer type but not the other, and that the gene:metabolite pair is cancer-type specific. Applying these models to all possible gene:metabolite pairs ( $N=4,640,646$ ) to compare different cancer subtypes in the NCI-60 cell lines yielded potentially relevant cancer-type specific gene:metabolite pairs. Results of this approach and comparison with other transcriptomics/metabolomics approaches will be discussed here.

**Poster #: 9**

**Abstract #: 2036**

**Abstract Title:** Fast alignment of peak lists

**Authors:** Ron Wehrens, Tom Bloemberg, Paul Eilers,

**Presenting Author Affiliation:** Wageningen UR

**Abstract Submission:**

Parametric Time Warping distorts the time axis of a chromatogram in order to maximize the overlap with a reference. The original version worked on complete time profiles only. However, often only peak positions are available, e.g., when comparing to data bases of known compounds.

Since 2015, the R package ptw implementing this technique now also supports aligning peak lists [1], e.g., coming from XCMS peak picking. It is shown that the technique is up to several orders of magnitude faster, since many irrelevant data points are no longer taken into account. An added benefit is that the careful preprocessing (in particular baseline subtraction) is nowhere near as important when aligning peaks - the peak picking method has made sure only the relevant information is available for the alignment algorithm. A final advantage of the new method is that wider time windows no longer take more time, and that this practically means that one does not have to fine-tune the algorithm for a particular data set.

We show examples of the use of ptw for a number of metabolomics experiments, using both LC-MS and LC-DAD data. This methodology will become ever more important with the increased access to FIAR data, allowing the simple and fast set up of restricted yet powerful time warping models. The R package, including demonstration data, is available from CRAN.

[1] R. Wehrens, T. Bloemberg and P. Eilers: Fast Parametric Time Warping of Peak Lists, Bioinformatics (2015)



**Poster #: 10****Abstract #: 2218****Abstract Title:** Batch correction for liquid chromatography - mass spectrometry data without the need for quality control samples**Authors:** Julie Wilson, Martin Rusilowicz, Michael Dickenson, Adrian Charlton, Simon O'Keefe,**Presenting Author Affiliation:** University of York**Abstract Submission:**

The need for reproducible and comparable results is of increasing importance in non-targeted metabolomics, especially when differences between experimental groups are small. Liquid chromatography - mass spectrometry (LC-MS) spectra are often acquired batch-wise so that necessary calibrations and cleaning of the instrument can take place. However, this may introduce further sources of variation, such as differences in the conditions under which the acquisition of individual batches is performed. Quality control (QC) samples are frequently employed as a means of both judging and correcting this variation. However, the use of QC samples can lead to problems. The non-linearity of the response can result in substantial differences between the recorded intensities of the QCs and experimental samples, making the required adjustment difficult to predict. Furthermore, changes in the response profile between one QC and the next cannot be accounted for and QC based correction can actually exacerbate the problems by introducing artificial differences. We introduce a "background correction" method that utilises all experimental samples to estimate the variation over time rather than relying on the QC samples alone. The method is compared with standard QC correction in terms of the reduction in differences between replicate samples and the potential to highlight differences between experimental groups previously hidden by instrumental variation.

**Poster #: 11**

**Abstract #: 2510**

**Abstract Title:** Pharmacokinetics-driven modelling of metabolomics data

**Authors:** Emilia Daghir-Wojtkowiak, Pawel Wiczling, Malgorzata Waszczuk-Jankowska, Roman Kaliszan, Michal Markuszewski,

**Presenting Author Affiliation:** Department of Biopharmaceutics and Pharmacodynamic

**Abstract Submission:**

Modelling of metabolomics data in targeted and untargeted approach is usually aimed at searching for potential disease indicators. Widely adopted multivariate approaches (PCA, PLS-DA, OPLS) however, are design to reduce dimensionality and perform multivariate analysis providing limited information on the usefulness of metabolites as potential disease indicators. Alternatively, metabolomics data can be fitted to the pharmacokinetics-based models allowing for more mechanistic characterization of collected data via natural incorporation of relationships between patient characteristics (age, gender) and model parameters to make inference about the disease status.

In this work we proposed the concept of pharmacokinetics-driven fully Bayesian approach to (i) model nucleosides/creatinine concentration ratios in a function of age, gender and case/control status and to further (ii) assess the posterior prediction of probability of cancer occurrence in an individual subject. The data set was randomly divided into training and validation set. Non-informative priors were used for modelling purposes. The model performance was evaluated using a posteriori predictive check. The validation set was used to evaluate the probability of cancer development for individuals with known nucleosides/creatinine concentration ratios, age and gender based on the developed model. The accuracy of classification was summarized by area under the ROC(AUROC), sensitivity and specificity.

As a consequence of cancer, methylthioadenosine concentration increased by a factor of  $\exp(1.82)((\exp(1.33)-\exp(2.47)))$ . Age influences nucleosides/creatinine concentration ratios for all nucleosides in the same direction which is likely caused by decrease of creatinine clearance with age. The individual a posteriori prediction of disease expressed via AUROC was 0.6 (0.51-0.69) with sensitivity and specificity of 0.63 (0.46-0.76) and 0.58 (0.45-0.68), respectively suggesting limited usefulness of nucleosides in predicting patient's disease status in this population. The widely adopted pharmacokinetics-based approach in drug development can be used to describe metabolomics data.

This work was supported by the grant funded by the National Science Center (2014/13/N/NZ7/00474)

**Poster #: 12**

**Abstract #: 2081**

**Abstract Title:** Statistical considerations in handling unwanted variation in large-scale metabolomics studies

**Authors:** Alysha De Livera,

**Presenting Author Affiliation:** The University of Melbourne

**Abstract Submission:**

Large-scale metabolomics studies often require the use of multiple analytical platforms, batches of samples, and laboratories, any of which can introduce a component of unwanted variation. In addition, every experiment is subject to within- platform and other experimental variation, which often includes unwanted biological variation. A notable difficulty in capturing the component of unwanted variation is that both the biological and experimental unwanted variation can be unobserved as well as observed. Although the removal of this unwanted variation is a vital step in the analysis of metabolomics data, it is considered a gray area in which there is a recognized need to develop a better understanding of the procedures and statistical methods required to achieve statistically relevant optimal biological outcomes.

In the statistical analysis of metabolomics data, the component of unwanted variation needs to be taken into account, in order to circumvent the problems of falsely identifying differentially abundant metabolites, having spurious correlations between metabolites, artificial clusters and poor classification. Recent studies have shown considerable success in inferring the unwanted variation from the data using control molecules. Here, we demonstrate how these methods, which rely on factor analysis, can be used in the context of metabolomics (i) to remove unwanted variation to obtain normalized data independently of the downstream statistical analysis [1], and (ii) to accommodate unwanted variation by directly incorporating terms into the statistical analysis which needs to be carried out[2]. The advantages and disadvantages of each approach, and the performance of these methods relative to widely-used metabolomics normalization approaches, are discussed through three metabolomics studies.

[1] De Livera, A. M., et al (2015). Statistical methods for handling unwanted variation in metabolomics data. *Analytical chemistry*, 87(7), 3606-3615.

[2] De Livera, A. M et al (2012). Normalizing and integrating metabolomics data. *Analytical chemistry*, 84(24), pp.10768-10776.

**Poster #: 13**

**Abstract #: 2349**

**Abstract Title:** Novel metabolite biomarkers of Huntington's disease (HD) as detected by high resolution mass spectrometry

**Authors:** Stewart Graham, Praveen Kumar, Ray Bahado-Singh, Andrew Robinson, David Mann, Brian Green,

**Presenting Author Affiliation:** Beaumont Health

**Abstract Submission:**

**Background:** Huntington's disease (HD) is a fatal autosomal-dominant neurodegenerative disorder affecting approximately 3-10 people per 100,000 in the Western World. The median-age of onset is 40 years, with death typically following 15-20 years later.

**Objectives:** In this study we aimed to biochemically profile post-mortem (PM) human brain excised from the frontal lobe and striatum of HD sufferers (n=14) and compared their profiles with controls (n=14). Our overarching goals were to identify potential central biomarkers of HD whilst providing an insight into the molecular basis of the disease by better understanding the biochemistry behind its progression.

**Methods:** We employed LC-LTQ-Orbitrap-MS for the global metabolite profiling of the polar metabolome from the striatum and frontal lobe regions.

**Results:** A total of 5,579 and 5,880 features were detected in the frontal lobe and striatum, respectively. An ROC curve combining two spectral features from frontal lobe had an AUC value of 0.916 (0.794 - 1.000) and following statistical cross-validation had an 83% predictive accuracy for HD. Similarly, two striatum biomarkers gave an ROC AUC of 0.935 (0.806 - 1.000) and following statistical cross-validation predicted HD with 91.8% accuracy. A range of metabolite disturbances were evident including but-2-enoic acid and uric acid which were altered in both frontal lobe and striatum. A total of 7 biochemical pathways (3 in the frontal lobe and 4 in the striatum) were significantly altered as a result of HD pathology.

**Conclusion:** This study highlights the utility of high-resolution metabolomics for the study of HD. Further characterization of the brain metabolome could lead to the identification of new biomarkers and novel treatment strategies for HD.

**Poster #: 14****Abstract #: 2423**

**Abstract Title:** A metabolomics approach for identification of novel biomarkers of chicken consumption

**Authors:** Xiaofei Yin, Helena Gibbons, Gary Frost, Lorraine Brennan, UCD

**Presenting Author Affiliation:** UCD

**Abstract Submission:**

A metabolomics approach for identification of novel biomarkers of chicken consumption

Xiaofei Yin, Helena Gibbons, Gary Frost, Lorraine Brennan

**Background:** Numerous studies have highlighted associations between meat intake and health outcomes. However, reliable and accurate dietary assessment methods are essential to confirm these proposed associations.

**Objective:** To identify and validate novel dietary biomarkers of chicken intake using a metabolomics approach.

**Materials and methods:** Urine samples from NutriTech Food Intake Study were used where volunteers consumed increasing amounts of chicken for three consecutive weeks. For example, females consumed 86 g/d in week 1, 176 g/d in week 2, and 308 g/d in week 3. The samples were analysed by a 600-MHz Varian NMR spectrometer and multivariate data analysis was performed with Simca-P software. Estimation of mean, standard deviation, and significant difference were calculated by SPSS. The putative biomarker was validated in a free-living population from National Adult Nutrition Survey (NANS). Receiver operating characteristic (ROC) was performed to evaluate the sensitivity and specificity of the biomarker.

**Result:** The application of PCA and PLS-DA models of postprandial and fasting urine samples revealed good separations between high and low chicken intake. Discriminatory regions were identified from the S-line plot. Examination of the NMR profiles led to the identification of guanidoacetate as a metabolite increased following chicken consumption. The concentrations of guanidoacetate in fasting urine samples significantly increased with increasing chicken intake ( $P < 0.001$ ), and were higher compared to the red meat group. ROC analysis to assess the classification ability of guanidoacetate between red meat and chicken groups represented a specificity and sensitivity of 0.90 and 0.98, respectively. The biomarker was confirmed in NANS cohort where chicken consumers had significantly higher concentrations of guanidoacetate ( $P < 0.001$ ), compared with non-consumers.

**Conclusion:** Guanidoacetate was successfully identified and confirmed as a biomarker of chicken intake by a metabolomics-based approach.

**Poster #: 15**

**Abstract #: 2133**

**Abstract Title:** FEATURE SELECTION METHODS FOR EARLY PREDICTIVE BIOMARKER DISCOVERY USING UNTARGETED METABOLOMIC DATA

**Authors:** Dhouha Grissa, Mélanie Pétéra, Marion Brandolini, Amedeo Napoli, Blandine Comte, Estelle Pujos-Guillot,

**Presenting Author Affiliation:** INRA, UMR1019, MAPPING, F-63000 Clermont-Ferrand

**Abstract Submission:**

Untargeted metabolomics is a powerful phenotyping tool for better understanding biological mechanisms involved in human pathology development and identifying early predictive biomarkers. However, this approach generates massive and complex data that need appropriate analyses to extract biologically meaningful information (Xi, 2014). In this context, this work consists in designing a workflow using knowledge discovery and data mining methodologies to propose advanced solutions for predictive biomarker discovery.

Data were collected from a mass spectrometry-based untargeted metabolomic approach performed on subjects from a case/control study within the GAZEL French population-based cohort. Different feature selection approaches were applied either on the original metabolomic dataset or on reduced subsets. The strategy was focused on evaluating a combination of numeric-symbolic approaches for feature selection with the objective of obtaining the best combination of metabolites, producing an effective and accurate predictive model. Relying first on numerical approaches, and especially on machine learning methods (SVM and RF) and on univariate statistical analyses (ANOVA), a comparative study was performed on the original metabolomic dataset and reduced subsets. The best k-features obtained with different scores of importance from the combination of these different approaches were compared and allowed determining the variable stabilities using Formal Concept Analysis.

The results revealed the interest of RF-Gini combined with ANOVA for feature selection as these two complementary methods allowed selecting the 48 best candidates for prediction. Using linear logistic regression strategy on this reduced dataset enabled us to obtain the best performances in prediction with a model including 5 top variables. Therefore, these results highlighted the interest of feature selection methods and the importance of working on reduced datasets for the identification of predictive biomarkers issued from untargeted metabolomics data. These data mining methods are essential tools to deal with massive datasets and contribute to elucidate complex phenomena associated with chronic disease development.

**Poster #: 16**

**Abstract #: 2144**

**Abstract Title:** msPurity: Assessment and Prediction of Precursor Purity for Mass Spectrometry Based Fragmentation in Metabolomics

**Authors:** Thomas Lawson, Ralf Weber, Martin Jones, Mark Viant, Warwick Dunn,

**Presenting Author Affiliation:** University of Birmingham

**Abstract Submission:**

Tandem Mass spectrometry (MS/MS) is a widely used approach to annotate and identify metabolites in complex biological samples. The importance of assessing the contribution of the precursor peak within an isolation window for MS/MS has been previously detailed in proteomics but to date there has been little attention paid to this data-processing technique in metabolomics. Here we present msPurity, a vendor independent R package for liquid chromatography (LC) and direct infusion (DI) MS/MS that calculates a simple metric to describe the contribution of the selected precursor peak to the population of isolated ions. What we call here “precursor purity” is calculated as per the Michalski approach (intensity of selected precursor divided by total intensity of the isolation window) with the exception that the metric is interpolated at the time of the MS/MS scan. The package was applied to Data Dependent Acquisition (DDA) based MS/MS metabolomics datasets derived from three metabolomic data repositories. For the ten LC-MS/MS DDA datasets with isolation windows less-than or equal to 1 +/- Da the median precursor purity score ranged from 0.61 to 0.85. The R package was also used to predict the precursor purity from an LC-MS dataset of a complex biological sample (*Daphnia magna*) using 0.5 +/- Da isolation windows, where the median predicted precursor purity score of the full width half maximum (FWHM) for all XCMS-determined features was 0.51. We therefore demonstrate that for complex samples there will be a large number of metabolites where traditional DDA approaches may struggle to provide reliable annotations.

**Poster #: 17****Abstract #: 2280**

**Abstract Title:** PeakForest: a spectral database and its toolbox, dedicated to the Metabolomics' community

**Authors:** Nils Paulhe, Christophe Duperier, Marie Lefebvre, Daniel Jacob, Pierrick Roger-Mele, Christophe Junot, Étienne Thévenot, Franck Giacomoni, Estelle Pujos-Guillot,

**Presenting Author Affiliation:** PFEM, UMR1019, INRA, Centre Clermont-Ferrand-Theix

**Abstract Submission:**

Introduction: Since 2013, MetaboHUB, the French infrastructure for metabolomics and fluxomics, provides tools and services to academic research teams and industrial partners in the fields of health, nutrition, agriculture, environment and biotechnology. One of its last developments is PeakForest.org, the MetaboHUB's reference spectral database. The first version of this tool already provides basic functionalities like a spectral database of standard compounds and querying / visualization tools plugged on it. The final version will include metabolome annotations of biological reference matrices.

PeakForest Database: the current release provides templates and a user-friendly interface in order to collect high quality spectral data (peak lists and metadata) from proton 1D-NMR and LC-MS instruments.

PeakForest's "toolbox": the web-application hosts two spectra viewers and external PeakMatching tools allowing a preview of stored data the real potential of PeakForest being its webservice access. This bot-friendly interface can be used by automated computer programming methods in high-throughput metabolomics context. "Workflow4Metabolomics" shall soon host tools using PeakForest as a resource. Any developer or bioinformatician can create his own program in order to interact with PeakForest.

Would you like your own PeakForest? Beyond MetaboHUB's reference spectra library, PeakForest is a Java environment you can use to create your own database! Thanks to virtualization techniques, we are able to quickly deploy and host new instances of PeakForest. For further information, please contact us!

Perspectives: PeakForest version 2.0. New functionalities including 13C-NMR, 2D-NMR, LC-MS/MS and GC-MS spectra management are scheduled. The PeakForest toolbox is ready to host services compliant with these data. At the end of 2016, this final version of PeakForest will provide storage and services for annotation of biological reference matrices.



**Poster #: 18****Abstract #: 2442****Abstract Title:** Don't reinvent the wheel! Re-using standards and software from computational proteomics**Authors:** Juan Antonio Vizcaino, Oliver Kohlbacher, Andrew Jones,**Presenting Author Affiliation:** EMBL-European Bioinformatics Institute**Abstract Submission:**

Data processing, management, and visualization are central and critical components in any mass spectrometry (MS)-based metabolomics experiment, and are often some of the most time-consuming steps, especially for labs without much bioinformatics support. The proteomics field has been developing data standards, and open source software libraries, analysis and visualisation tools, which we believe can be extended and adapted to the needs of computational metabolomics. We would like to engage the metabolomics community in joint developments profiting researchers from proteomics and metabolomics alike.

In some cases, this is already happening (e.g., the mzML data standard for MS and related tools such as ProteoWizard), and there are initiatives such as the “Computational MS” group that try to “fill the gap” between both fields (<http://compms.org/>). Furthermore, existing data standards such as mzTab and mzQuantML and the related open source software libraries such as the ones developed by our groups, namely jmzTab, jmzReader (for MS formats, including mzML and peak lists), ms-data-core-api, jmzQuantML, and mzqLibrary could be extended to properly support MS metabolomics data, and some prototypes have been started in this space. For instance, mzTab has already been mocked-up for reporting metabolomics and glycomics results. In addition, existing visualisation stand-alone tools such as PRIDE Inspector (<https://github.com/PRIDE-Toolsuite/pride-inspector>) or different web components could also be extended and reused. Another example to highlight is the OpenMS framework, which comes with a variety of pre-built and ready-to-use tools for proteomics and metabolomics data analysis (TOPPTools) and powerful 2D and 3D visualization (TOPPView), and that can be used to build data analysis workflows.

To summarize, we would like to see an increasingly closer collaboration between both fields to avoid duplication of efforts – and such efforts may be coordinated via international consortia such as Proteomics Standards Initiative, ProteomeXchange, COSMOS and the Metabolomics Standards Initiative.

**Poster #: 19**

**Abstract #:** 2535

**Abstract Title:** SIRIUS coupled with CSI:FingerID: an approach for the structural elucidation of unknowns using tandem ms

**Authors:** Kai Dührkop, Marvin Meusel, Juho Rousu, Sebastian Böcker,

**Presenting Author Affiliation:** Friedrich-Schiller-University

**Abstract Submission:**

Tandem mass spectrometry is a predominant technique for high-throughput metabolomics experiments. Whereas it is possible to detect thousands of metabolites simultaneously from a biological sample, identifying these metabolites remains a challenging task. Here, we present the newest version of SIRIUS, a framework for the automated analysis of tandem MS experiments. It combines isotope pattern analysis and fragmentation pattern analysis using fragmentation trees (FT). A FT assigns molecular formulas to each signal peak in the fragmentation spectrum, and connects these with hypothetical fragmentation reactions. FTs are idealized representations of MS/MS spectra, containing neither noise peaks nor mass errors. Tools for structure elucidation (MetFrag, MAGMa, CSI:FingerID) show improved performance when using FTs instead of MS/MS spectra. In particular, CSI:FingerID's search performance increases by 65 % when using FTs.

The new SIRIUS 3.3 comes with a revised isotope pattern analysis, an automated approach for detecting the chemical elements of the compound's formula, and a new graphical user interface. Its open API allows an easy integration of SIRIUS into other frameworks (OpenMS or MZmine2).

CSI:FingerID, so far only available as web application, is now integrated into SIRIUS and can be used to batch-process measurements in

large scale. The graphical user interface visualizes predicted structures and identified compounds. SIRIUS computes FTs solely from MS/MS data without using any database, making it the tool of choice for dealing with so-called 'unknown unknowns' - compounds that are not even contained in structure databases like PubChem. SIRIUS coupled with CSI:FingerID can predict the molecular formula as well as the presence or absence of certain substructures and side-groups of such unknown unknowns and suggest structural similar compounds from a structure database. Thus, it offers an interactive approach for structure elucidation of unknown unknowns, and an automated approach for compounds that are contained in biological structure databases.

**Poster #: 20**

**Abstract #: 2270**

**Abstract Title:** Statistical Metabolomics: new computational tools for metabolomics data analysis

**Authors:** Francesco Del Carratore, Andris Jankevics, Simon Rogers, Rainer Breitling,

**Presenting Author Affiliation:** Manchester Institute of Biotechnology, Faculty of

**Abstract Submission:**

One of the most widely used analytical techniques in metabolomics is mass spectrometry, usually coupled with a separation system such as liquid chromatography (LC). In a typical mass spectrometry-based metabolomics experiment two different conditions are compared in order to find the biological differences between two (or more) conditions. The huge amount of complex data generated by mass spectrometry needs automated tools for the extraction of useful biological information. In particular, the identification of differentially expressed features and their annotation (the association of the experimental features to specific metabolites) play a pivotal role in such analysis. Both these challenges have been tackled in this project.

1. Regarding the identification of differentially expressed features, the widely used Rank product method has been refactored allowing a more reliable application to metabolomics datasets.
2. The incorporation of information about the possible relationships between the empirical formulas to assign to mass peaks provides better performance than annotation based on mass alone. Considering this, a Bayesian method capable of incorporate information about possible biochemical transformations, relationships between adducts and relationships between isotopes has been developed, which will provide significant improvement to the annotation process.

Both of these methods are being implemented in the mzMatch data analysis pipeline, to increase both the reliability and reproducibility of the data interpretation process in metabolomics experiments and to facilitate their integration in a general systems-biology modelling strategy informed by metabolite profiling data. Ultimately, this work will be part of a synthetic biology pipeline, in which metabolite profiling data are feeding directly into computational modelling tools to drive the design and engineering of microbial cells with novel useful functions.

**Poster #: 21****Abstract #:** 2496**Abstract Title:** A prior knowledge-based computational workflow for de novo structural elucidation of small molecules in mass spectrometry metabolomics**Authors:** Daniel Taylor, Yingjie Ji, Ralf Weber,**Presenting Author Affiliation:** University of Birmingham**Abstract Submission:**

Structural annotation of metabolites in untargeted mass spectrometry (MS)-based studies remains a major challenge. Despite significant progress over recent years in expanding compound databases and mass spectral libraries, a large proportion of metabolite features detected remain unidentified. This is particularly true for MS experiments that typically measure poorly annotated metabolomes. Previously, structure elucidation tools (e.g. OMG and MOLGEN) have been developed and applied to expand the search space, however they produce a huge number of implausible chemical structures, which results in high numbers of incorrect and false-positive assignments. Furthermore, databases of in silico enzymatically synthesised metabolites have been created (e.g. IIMDB and MINEs) to help address the problem discussed here. Nonetheless, improved approaches are required to increase the size of the metabolic search space, ideally in a controlled manner that leverages knowledge from biology.

Here, we have developed a Python-based workflow to generate de novo structures using prior knowledge (e.g. atom-bond connectivity and structural features) from existing compound databases and libraries. The freely-available workflow has been validated using classification and 'likeness' score calculations, benchmarked against existing tools and resources, and applied to untargeted studies (i.e. liquid chromatography and direct infusion based-MS and MS<sub>n</sub>).

Preliminary findings include that the workflow 1) correctly generates endogenous metabolites (ca. 5000,  $\geq 400$ Da, containing C<sub>6</sub>H<sub>8</sub>N<sub>2</sub>O<sub>2</sub>P<sub>2</sub>S<sub>2</sub>) present within HMDB 2) reduces the number of implausible structures generated up to ca. three orders of magnitude in comparison to other structure generators 3) correctly annotates spectral trees ( $\geq$ MS3) of ca. 150 (known) compounds of diverse chemistry (with zero or relative small number of false positives). Note: prior knowledge of the compound of interest (or structurally highly similar compound) has not been included in the elucidation process to avoid bias. We believe this workflow provide an important step towards improved metabolite annotation.

**Poster #: 22**

**Abstract #: 2230**

**Abstract Title:** Non-targeted LC-MS-based lipidomics with OpenMS in KNIME

**Authors:** Fabian Aicheler, Oliver Kohlbacher,

**Presenting Author Affiliation:** Center for Bioinformatics, University of Tuebingen

**Abstract Submission:**

Lipidomics as a branch of metabolomics is steadily increasing in importance. Biomedical example applications include mechanistic studies and biomarker discovery for various diseases like diabetes, cancer, or neurodegenerative diseases. As in metabolomics, LC-MS offers high sensitivity and a large dynamic range to lipidomics, lending itself to profiling and identification. In light of the large data volumes observed in commonly occurring high-throughput studies, automated processing is a necessity.

Here we present a fully automated processing pipeline for lipidomics. The pipeline focusses around a core of tools from OpenMS, an open-source library and framework for computational mass spectrometry methods. Its large toolset includes methods for small molecule mass trace detection, isotopic trace aggregation and quantitation of (lipid) ions. It further supports alignment of temporal shifts between runs and the determination of corresponding features across samples. Our lipidomics pipeline is realized in the graphical workflow engine KNIME. The comprehensive integration of OpenMS tools therein allows for interactive MS data exploration and statistical analysis, enabling large-scale profiling of lipidomics data. Lipid identification is supported by OpenMS tools combining searches by accurate mass and spectral similarity. Included prediction models for lipid retention time allow for an automated pruning of putative identifications. The refinement of lipid identifications via such a guided analysis facilitates possible manual evaluation. OpenMS is released under a BSD 3-clause open-source license on the OpenMS website ([www.OpenMS.de](http://www.OpenMS.de)). Our pipeline will be available there as well. OpenMS nodes and all other nodes used in the pipeline are freely available in KNIME.

We thus provide the integration of lipidomics analysis steps ranging from feature detection and quantification to statistical analysis and identification with OpenMS in KNIME. Our pipeline enables automated large-scale profiling and identification of lipidomics data using accurate mass search, spectral similarity, and retention time prediction-based candidate ID filtering.

**Poster #: 23**

**Abstract #: 2529**

**Abstract Title:** Gap Filling of GC-MS Data Matrices using PyMS

**Authors:** Sean O'Callaghan, David De Souza, Malcolm McConville,

**Presenting Author Affiliation:** Metabolomics Australia

**Abstract Submission:**

PyMS (Python Mass Spectrometry) is a Python library for Gas-Chromatography Mass-Spectrometry (GC-MS) data processing. PyMS is commonly used for routine untargeted metabolomics GC-MS experiments and provides a simple, automated pipeline for unsupervised data processing. A typical pipeline for a PyMS GC-MS data processing task involves data filtering, peak picking and peak alignment. The final output is a data matrix which provides an area for each metabolite present across each of the samples in the experiment.

The presence of missing values in the data matrix increases the variance of the data, which has a detrimental effect on subsequent data analysis. Missing values can result from actual absence of a compound from a sample, errors in peak picking and errors in alignment. Many statistical packages use imputation of small values, sometimes random or related to a low value in the data matrix to replace these missing values to allow statistical analysis.

We present an algorithm for replacing missing values with values mined from the original mass spectrometry data. In many cases, where errors in peak picking or alignment have taken place, a re-integration of a real chromatographic peak takes place, providing additional accuracy in the data matrix. In a small minority of cases, where a compound is truly not detected by the mass spectrometer, random noise on the particular ion channel is integrated providing a small random value for imputation in the data matrix.

Typically the rate of missing values of a standard GC-MS data processing task in PyMS is 20-30%. The new PyMS GapFill algorithm reduces this to 2-10% depending on the nature of the experiment. This results in more accurate reporting of metabolite values and greatly improves the quality of statistical analysis.

**Poster #: 24**

**Abstract #: 2576**

**Abstract Title:** Scalable analysis setup enables high-throughput metabolomics

**Authors:** Stephanie Herman, Marco Capuccini, Payam Emami Khoonsari, Anders Larsson, Kim Kultima, Ola Spjuth,

**Presenting Author Affiliation:** Dept. of Medical Sci., CARAMBA, Uppsala University

**Abstract Submission:**

There exists a wide range of e-infrastructure and data analytics tools in industry. The music industry uses it to store and distribute music all over the world. The advertising and marketing industry use it to extract information derived from customers' buying patterns. These frameworks and analytics tools are not novel, they are pre-developed and highly functional. There is a great opportunity for the metabolomics community to take advantage of them for data analysis.

In order to assess the current bottlenecks in metabolomics, lying mainly in data management, we suggest the use of cloud computing resources in combination with a microservice-based architecture style. The microservices architecture style is where complex applications and workflows are divided into isolated and smaller services. These constricted processes are independently deployable and compatible with one another, like building blocks of Lego. This kind of software design enables these blocks of services to be combined in multiple ways, creating pipelines or workflows of actions.

Within the PhenoMeNal H2020 project we have implemented a scalable R-based proof-of-concept workflow, using MANTL (by Cisco Cloud). The workflow can be run on any commercial or in-house cloud and controlled from any computer, and demonstrates the utility of virtual infrastructures for scalable and interoperable metabolomics analysis.



**Poster #: 25**

**Abstract #: 2028**

**Abstract Title:** The Standard Fly: A recommended addition to all Drosophila metabolomics studies.

**Authors:** Laura Reed,

**Presenting Author Affiliation:** University of Alabama

**Abstract Submission:**

One limitation to the broad utility of metabolomics studies is a lack of standardization of methods and data interpretation across analysis facilities even while the number of metabolomics studies are increasing rapidly. While the metabolomics community works toward broadly applied standardization recommendations there is the immediate need for resources to facilitate comparisons across studies. Toward this goal, we have developed the Standard Fly to be included in all metabolomics experiments in the model organism *Drosophila* (the fruit fly), as recommended by the International *Drosophila* Metabolomics Curation Consortium. The Standard Fly is the Oregon-R genetic line used in the modENCODE project (Bloomington Stock Number 25211) raised to adulthood under highly-controlled conditions on a cornmeal-molasses diet. Several hundred samples of 3-5 day old mated adults (15 males and 15 females, ~ 30 mg of tissue) were snap frozen at one time. Future batches will be generated under identical conditions as needed. Here we report the metabolome properties of the Standard Fly as determined by GC-MS and NMR analyses, and how it compares to other genotypes, dietary treatments, and related-species. Samples are available free of charge to any lab wishing to include them in their metabolomics studies. We also encourage researchers to include the Oregon-R-modENCODE genetic strain in their specific experimentally manipulations, also available upon request. By including this common sample across studies, we will be better able to compare diverse datasets. Correspondingly, we recommend other model organism metabolomics research communities consider establishing a community standard specific to their needs.

**Poster #: 26**

**Abstract #: 2185**

**Abstract Title:** Recent developments in the MetaboLights database

**Authors:** Kenneth Haug,

**Presenting Author Affiliation:** EMBL-EBI

**Abstract Submission:**

With the international success of the MetaboLights repository, EMBL-EBI is now focusing efforts into adding further value and functionality. In addition to general enhancements to the current infrastructure, EMBL-EBI is working on integrating analysis tools into MetaboLights Labs. Here we will introduce online integrated data analysis tools. The first notable integrations are LipidHome and MetaboAnalyst. The large amount of publicly available primary research data in MetaboLights, an average size of 20Gb per study, is ideal for further analysis and refinement. MetaboLights is experiencing a very rapid growth, clearly serving the community in a proactive manner. Currently, MetaboLights has about 300 complete metabolomics experiments. In addition to this, there are some 22000 reference metabolites with detailed information about the chemistry, pathways, reactions, spectral references and literature. There are about 2100 different organisms reported from studies and reference compounds. MetaboLights is endorsed by journals, like Nature Scientific Data, PLOS and Metabolomics, and fully supports secure private access to restricted or pre-publication datasets. EMBL-EBI initiated and is a founding member of MetabolomeXchange.org, an open portal for publishing, searching and identifying open access metabolomics datasets. All public data in MetaboLights is freely downloadable for any purpose, and we continue to advocate open data access.

**Poster #: 27**

**Abstract #: 2240**

**Abstract Title:** Data Quality Task Group Identifies Community Practices

**Authors:** Dan Bearden, Richard Beger, Mark Viant, Robert Trengove, Claude Guillou, Art Edison, Warwick Dunn,

**Presenting Author Affiliation:** NIST

**Abstract Submission:**

**Objectives:** The Metabolomics Society Data Quality Task Group (DQTG) developed a questionnaire to provide baseline information about current quality assurance (QA) and quality control (QC) practices applied in the metabolomics community. Although it is sometimes difficult to come to a consensus about what constitutes QA/QC, most recognize that these are practices that are intended to improve the consistency and veracity of the analytical measurements in untargeted metabolomics studies. For the purposes of this exercise, QA was defined as the set of activities that a laboratory performs IN ADVANCE OF OR IN PREPARATION FOR analysis of samples for a project that are applied to improve data quality in projects. QC was defined as the set of procedures or measurements which allow the review of factors involved in collection and analysis of the data and activities that a laboratory does during or immediately after analysis for a project that are meant to DEMONSTRATE the quality of project data or to IDENTIFY analytical errors or mistakes.

**Outcomes:** We received 97 individual responses from 84 institutions covering NMR, LC-MS, GC-MS, and other analytical technologies in all fields of metabolomics. There was a vast range of responses concerning the use of QA and QC approaches. The DQTG QA/QC questionnaire showed the QA and QC use was not uniform across metabolomics labs and there is a need to establish minimum standards in the use of QA and QC measurements and reporting in metabolomics.

**Poster #: 28****Abstract #: 2241****Abstract Title:** Metabolomics-Based Pre-Analytical Quality Control of Human Plasma Samples**Authors:** Laura Schnackenberg, Lisa Pence, Jinchun Sun, Thomas Schmitt, Zhijun Cao, Li-Rong Yu, Thomas Gerken, Beate Kamlage, Sandra Gonzalez-Maldonado, Bianca Bethan, Peter Driemert, Philipp Schatz, Richard Beger,**Presenting Author Affiliation:** NCTR - US FDA**Abstract Submission:**

**Introduction:** Highest sample quality is one key for successful research in the healthcare area and is particularly important in projects which involve OMICS platforms or a systems biology approach. The objective of our study was to analyze how pre-analytical steps like time to processing, temperature of blood or plasma, or the centrifugation force applied impact the metabolome of human EDTA plasma samples and consequently decrease the credibility and validation of data in the area of biomarker development.

**Methods:** Six tubes of EDTA blood samples were obtained from 20 healthy volunteers and processed to plasma by applying five defined pre-analytical confounding factors in addition to a control. All samples were analyzed by mass-spectrometry based metabolomics (MxP® Broad Profiling, MxP® Catecholamines, MxP® Lipids (sphingoids), and MxP® Eicosanoids) and data for 254 metabolites derived. Further, a targeted assay developed for the purpose of plasma sample quality control was applied (MxP® Quality Control Plasma). Statistical analysis was done by a mixed linear model and the false discovery rate was calculated by the method created by Benjamini & Hochberg.

**Results:** The metabolite classes of eicosanoids, catecholamines, and sphingoids showed the highest response towards pre-analytical variation. Among the confounders applied, blood storage at room temperature for six hours prior to centrifugation had the highest impact with 36% of the plasma metabolites significantly changed ( $p < 0.05$  and false discovery rate  $< 0.2$ ). The quality control assay (MxP® Quality Control Plasma) detected plasma samples of a poor pre-analytical quality due to prolonged storage of blood or plasma with high sensitivity and specificity.

**Conclusion and outlook:** High-level interpretation of -omics results requires a comprehensive knowledge of the impact of the pre-analytical phase on the results and their underlying physiological and chemical mechanisms. The next steps will be peptidomics and proteomics analysis of the same samples and a data integration analysis.

**Poster #: 29****Abstract #: 2538****Abstract Title:** The ISA framework evolution: serialisations, browser, API and fluxomics configurations**Authors:** Philippe Rocca-Serra, Alejandra Gonzalez-Beltran, Massimiliano Izzo, David Johnson, Susanna Sansone, Kenneth Haug, Jose-Ramon Macias, Reza Salek, SIRM WG,**Presenting Author Affiliation:** University of Oxford e-Research Centre**Abstract Submission:**

The ISA framework for standards-compliant data collection, curation, management and publication encompasses a model, several serializations options, a growing set of collaboratively developed software components. Adopted by an expanding range of different resources, including the EMBL-EBI MetaboLights and Springer Nature's Scientific Data journal, ISA enables the production of Findable, Accessible, Interoperable, Reusable (FAIR) datasets. This update introduces an array of new feature supporting this vision.

For creation and manipulation of ISA compliant dataset, a new set of programmatic calls are now available via the Python programming language. The ISA tools API (<https://github.com/ISA-tools/isa-api>) and associated RESTful service allows developers to programmatically create, load, and export ISA content, as well as convert between the ISA tabular and JSON formats. Function are available to convert to/from other formats such as SRA XML and Metabolomics Workbench (MWTAB), supporting metabolomics and functional genomics experiments. The ISA API can also retrieve ISA configurations (sets of XML documents describing assay specific workflow patterns) from a Github-based repository, where versioned releases are kept (<https://github.com/ISA-tools/Configuration-Files/releases>).

The API is being expanded to convert native Biocrates XML documents to ISA formats and facilitate deposition to MetaboLights, greatly speeding up the publication of targeted metabolomics datasets. An ingester is also being developed allowing ISA-Tab representation of datasets from the NIH Metabolomics Workbench, the US counterpart to MetaboLights, a stepping stone to future data exchange. Finally, a new set of configurations are now publicly available to support reporting of Stable Isotope Resolved Metabolomics and Fluxomics studies.

For ISA metadata presentation, the ISA-explorer component offers cost-effective means to browse and search dataset experimental metadata. Built around proven web technologies (HTML/CSS/JavaScript), a web browser is all that is needed an exemplar implementation in Scientific Data is available at: <http://scientificdata.isa-explorer.org>.

**Poster #: 30****Abstract #:** 2540**Abstract Title:** How close are we to metabolomics data analysis reproducibility? Data sharing, data standards and workflows for metabolomics**Authors:** Reza Salek, The W4M Core Team, The MetaboLights Team, The COSMOS consortium,**Presenting Author Affiliation:** EMBL-EBI**Abstract Submission:**

With increasing amounts of metabolomics publications and data being produced, only a small portion of datasets are publically shared. For metabolomics results to become reproducible mere descriptions of investigations as text in a manuscript are not sufficient. What can increase the chance of reproducibility, the ultimate aim within any scientific field is to have a standards framework for data sharing and reporting results as well as sharing the complete study files. In recent years, metabolomics data standards have developed extensively, to include the primary research data, derived results and the experimental description and importantly the metadata in a machine-readable way. This also includes vendor independent data standards such as mzML for mass spectrometry and nmrML for NMR raw data that have both enabled the development of advanced data processing algorithms by the scientific community. In addition, there has been recent efforts in creating metabolomics data analysis workflow potentially producing auditable trail for data analysis reproducibility. Altogether, all this should pave the way for both reproducible research and data reuse, in metabolomics.

**Poster #: 31**

**Abstract #: 2451**

**Abstract Title:** Standardizing Lipidomics: Results of a NIST Interlaboratory Comparison

**Authors:** John Bowden, Alan Heckert,

**Presenting Author Affiliation:** National Institute of Standards and Technology

**Abstract Submission:**

A common problem of emerging disciplines, such as lipidomics, is a lack of standardized protocols, often leading to a large variability when comparisons are made between analyses and/or laboratories. Thus, a need has been realized to standardize lipidomic methodologies in such a way to harmonize measurement within the lipidomics community. The National Institute of Standards and Technology (NIST) has been conducting an interlaboratory comparison exercise for lipidomics using human-based serum and plasma Standard Reference Materials (SRMs). To date, 27 national and international laboratories have participated and span across all lipidomic arenas. The exercise was designed to highlight: 1) how much variance in measurement was present within the lipidomics community, 2) which lipids were present in the SRMs, 3) whether SRMs were needed in lipidomics, 4) challenges present with current lipidomic measurement, and 5) potential optimal protocols for measurement of specific lipids/lipid classes.

The SRMs sent to each laboratory included SRM 2378, Fatty Acids in Frozen Human Serum (levels 1, 2, and 3) and SRM 1950 Metabolites in Frozen Human Plasma. Each participating laboratory was asked to use the analytical procedures that they typically use in their laboratories (in triplicate) and to report the data for those lipids that they typically identify and quantify with confidence. Over 1000 lipids were identified across all laboratories for each material. Consensus values (with uncertainty) were calculated and evaluated for those lipids measured by three or more laboratories. The performance of each laboratory was determined (in relation to the consensus mean) and compared with the employed lipidomic methodology provided by the laboratories to help pinpoint specific problematic areas with current lipidomic measurement (e.g., false positives, diverse quantitation strategies). In addition to highlighting the magnitude of lipid measurement (dis)agreement, the critical role of reference materials for improved community-wide lipidomics measurement will also be discussed.

**Poster #: 32****Abstract #: 2352****Abstract Title:** Metabolomics profiling related to circulating folic acid among population with mandatory fortification**Authors:** Josiane Steluti, Antonio Carioca, Alexsandro Silva, Aline Carvalho, Andreia Miranda, Ismael Silva, Regina Fisberg, DIRCE MARCHIONI,**Presenting Author Affiliation:** School of Public Health, University of Sao Paulo**Abstract Submission:**

Background: High oral doses of folic acid have been shown to bypass the normal folate absorption mechanism, resulting in existing levels of circulating folic acid (FA), in special, after mandatory fortification of flour with FA in many countries. FA is a key component of one-carbon metabolism which some amino acids are involved. This metabolism is essential to metabolic pathways as hundreds of intracellular transmethylation reactions, including DNA methylation and DNA synthesis. However, the knowledge about the metabolism and biological effects of circulating FA is lacking. Objective: To identify the metabolomics profiling and its association with circulating FA. Methods: Data was from the "Health Survey of Sao Paulo" (ISA-Capital2008), a population based cross-sectional survey in Sao Paulo, among 169 individuals aged 20 years or older. Plasma samples were assayed to circulating FA using Affinity/HPLC with electrochemical detection method. The metabolites were determined by mass spectrometry of the targeted type, and 21 amino acids were considered. Metabolomics profiling were performed using factor analysis with extraction of principal components by varimax rotation, and regress models were used to investigate the relationship between metabolomics profiling and circulating FA. Results: The mean of age was 50.6 y (95% CI: 47.7-53.5), and women accounted for 58.2% of the sample. Five profiling (factors) were retained, and the composition of factors were: factor 1 (Isoleucine, Leucine, Lysine, Methionine, Phenylalanine, Tryptophan, Tyrosine, Valine), factor 2 (Asparagine, Glycine, Serine, Threonine), factor 3 (Arginine, Glutamate, Histidine), factor 4 (Citrulline, Glutamine, Ornithine) and factor 5 (Alanine, Aspartate, Proline). Only the metabolites of factor 3 were associated with the higher tertile of circulating FA after adjusted for sex, age and BMI ( $\beta=0.64$  95%CI 0.27-1.00). Conclusion: Metabolomic profiling (Arginine, Glutamate, Histidine) was associated with circulating FA that may aid in understanding of possible alterations in amino acids metabolism due to excessive dietary intake of FA.



**Poster #: 33****Abstract #: 2344****Abstract Title:** MetaboLights: a reference repository for METASPACE**Authors:** Jose Macias, Reza Salek, The MetaboLights Team,**Presenting Author Affiliation:** EMBL-EBI**Abstract Submission:**

One of the challenges to be addressed by the European Horizon2020 project METASPACE on Bioinformatics for Spatial Metabolomics (<http://metaspace2020.eu>) is to maximise the use of new, diverse and complex data delivered by High Resolution Imaging Mass Spectrometry (HR imaging MS). HR imaging MS usually produce large (10-500 GB), complex (10.000.000 images / 10.000-100.000 spectra), and information-rich data potentially representing thousands of metabolites together with their spatial distribution within a sample. Turning such data into meaningful molecular information requires the use of powerful bioinformatics tools for metabolite annotation and identification of those MS signals and representing the results in light of existing molecular and biological knowledge.

An open online engine for metabolite annotation of HR imaging MS is being developed in METASPACE (see the abstracts by Theodore Alexandrov and Andrew Palmer) providing easy access to current and novel automated analysis methods. This engine will be integrated with different metabolic databases and online services. MetaboLights (<http://metabolights.org>), the first open access, general-purpose database for metabolomics is considered to be a key reference repository for the METASPACE project by hosting all spatial metabolomics data and metadata being produced and by integrating the METASPACE bioinformatics services with MetaboLights in the future.

What is important for hosting big data from HR imaging MS coming from different labs worldwide, MetaboLights is planning to upgrade upload mechanisms. While FTP has been successfully used for uploads of gigabytes of data into MetaboLights already, the requirements for new data sets (in the range of 10-500 GB in size per study) would make FTP impractical, so other alternatives are being implemented. Initially, the following services for high-speed transfer of large data sets over the network will be considered: Aspera (<http://asperasoft.com>) and Globus (<https://www.globus.org/>), as they are also in use by other genomics and proteomics services at EMBL-EBI.

**Poster #: 34****Abstract #: 2575****Abstract Title:** A robust mass spectrometry-based untargeted metabolomic method and quality control procedure for epidemiological studies**Authors:** Pekka Keski-Rahkonen, Agneta KISS, Joseph Rothwell, Nivonirina Robinot, Augustin Scalbert,**Presenting Author Affiliation:** IARC**Abstract Submission:**

Major challenges in applying untargeted mass spectrometry-based metabolomics to epidemiological studies are the large numbers of samples that are required for well-powered epidemiological studies, and the high reproducibility that is needed to enable detecting minute changes in metabolite levels. Common analytical problems include the drift in response over time, and variability associated with the handling of small-volume samples. Some post-acquisition data adjustment can be performed, but acquiring high quality raw data is critical. However, due to the untargeted nature of the analysis, assessing the data quality can be difficult.

Here we report an untargeted method with a robust quality control (QC) procedure that has been specifically set up to support the analysis of up to 600 samples/batch in epidemiological studies. Plasma/serum samples (20-30  $\mu$ L) are prepared by protein precipitation in well plates and analyzed by UHPLC-QTOF. A 13-minute analysis time enables a throughput of up to 600 samples per week. QC procedure includes a system suitability test before the analytical batch, individually extracted study-specific QC samples (injected after every 12 samples) and blanks, and a repeatedly analyzed laboratory control sample for monitoring instrument performance.

Within-run precision of the method for ten known plasma metabolites was 4.0-6.6% (CV, 20 repeated injections). Representative results for a batch of 499 plasma samples showed 3012 features with CV less than 20% and present in 90% of the 43 QCs. The same ten known metabolites in the QCs had CVs of 7.6-14.4%, with retention time variability less than  $\pm 0.019$  min. Well plate specific blanks enabled reliable removal of background features, and using a laboratory control sample made it possible to assess the compatibility of the data with that from preceding and following batches. Examples of successful applications to cross-sectional, nested case-control, and nutritional intervention studies will be presented, demonstrating suitability for epidemiological investigations.

**Poster #: 35**

**Abstract #: 2347**

**Abstract Title:** Boosting compound identification confidence by exploiting all HRAM spectral information: Integrating accurate mass, true isotopic pattern, in-source fragmentation, MS/MS fragmentation, and retention time

**Authors:** Nikolas Kessler, Stephan Maevers, Frederik Walter, Marcus Persicke, Jörn Kalinowski, Matthias Szesny, Aiko Barsch, Heiko Neuweiger,

**Presenting Author Affiliation:** Bruker Daltonik GmbH

**Abstract Submission:**

Confident compound identifications is still one of the major bottlenecks in metabolomics. While there are ongoing efforts to refine the definitions and levels of metabolite identifications that were first proposed by the MSI initiative, it is clear that higher levels of identification confidence can be reached by joining accurate measurement technology, orthogonal molecular features, and sophisticated software tools. Here we present a single integrated software solution for pushing the confidence in identifications at different levels: molecular formula, compound class, structure, or verified targeted identification. This highly integrated functionality is implemented in a new version of the MetaboScape® software.

We could highly improve the quality of compound identifications in a study investigating the arginine biosynthesis in *Corynebacterium glutamicum* conducted by HRAM LC-QTOF non-targeted metabolomics. The integrated tools in MetaboScape were used to create annotations throughout increasing confidence levels: First, for all compound spectra molecular formulas were generated based on accurate masses, true isotopic patterns, and in-source fragmentation patterns, applying metabolomics-tailored rules and filters. Afterwards, public chemical databases were queried to find structural candidates for the generated molecular formulas of interesting features. Then, a customized analyte target list was applied to additionally exploit retention times of expected features. Lastly, MS/MS spectral library comparisons and in-silico fragmentations (MetFrag [1,2]) enabled to create and verify identifications based on MS/MS fragmentation patterns.

The outlined strategy will enable users to achieve highest confidence in compound identifications based on LC-HRAM-MS/MS spectral information using an integrated “turnkey” solution.

[1] Wolf S., et al. BMC Bioinformatics 2010, 11: 148

[2] Ruttkies C., et al. Journal of Cheminformatics 2016, 8:3

**Poster #: 36**

**Abstract #: 2252**

**Abstract Title:** Metabolic Changes of Radiation-Exposed Rat Liver

**Authors:** Chang-Mo Kang,

**Presenting Author Affiliation:** Korea Institute of Radiological & Medical Sciences

**Abstract Submission:**

NMR-based metabolic profiling was used to study the influence of ionizing radiation on the parameters of the metabolite markers of rat liver model system. We applied whole-body X-ray irradiation to the rat at the dose of 6Gy, and profiled metabolites by 2D nuclear magnetic resonance (NMR) spectroscopy to sort out candidate molecules that can be used to deduce the required information. We identified 32 metabolites from rat liver extracts. Statistical analysis suggested that samples with or without X-ray irradiation and samples harvested 24 or 72 h after irradiation could be divided into four quadrants of the principal component space. In conclusion, this work can establish a base for classifying the radiation-exposed patients.

**Poster #: 37**

**Abstract #: 2254**

**Abstract Title:** Application 2D-NMR Spectroscopy on Radiation Exposed Human Primary Cells

**Authors:** Sung Hee Hong,

**Presenting Author Affiliation:** Korea Institute of Radiological & Medical Sciences

**Abstract Submission:**

We employed the primary cell model system as a first step toward establishing a method to assess the influence of ionizing radiation by using a combination of common and abundant metabolites. We applied X-ray irradiation at the amounts of 0, 1, and 5 Gy to the human primary fibroblast cells and then were harvested 24, 48, or 72 h later, and profiled metabolites by 2D-NMR spectroscopy to sort out the candidate molecules that could be used to distinguish the samples under different irradiation conditions. We traced metabolites stemming from the input  $^{13}\text{C}$ -glucose, identified twelve of them from the cell extracts, and applied statistical analysis to find out that all the metabolites, including glycine, alanine, and glutamic acid, were increased upon irradiation. The combinatorial use of the selected metabolites showed promising results that the product of signal intensities of alanine and lactate could differentiate samples according to the dose of X-ray irradiation. In conclusion, this work can establish a base for classifying the patients exposed to radiation.

**Poster #: 38****Abstract #: 2087****Abstract Title:** Effects of Repeated Exposure to Maleic Acid in Rats by <sup>1</sup>H NMR-based Metabolomics**Authors:** chi-hung Chen, Ching-Yu Lin, Charlene Wu, Kuen-Yuh Wu,**Presenting Author Affiliation:** national taiwan university**Abstract Submission:**

Food safety are noticed by the public. In 2013, the Department of Health (DOH) in Taiwan declared that some starch-processed foods were illegally added food additive, maleic acid/maleic anhydride. Modified starch can enhance favorable properties, such as viscosity, texture, and elasticity in food. Accidental consumption of maleic acid at low levels does not cause significant adverse health effects however, long term exposure of high levels of maleic acid can induce kidney damage. The molecular effects of repeated maleic acid exposure are still largely unknown. In this study, we intend to understand metabolic effects of repeated exposure to maleic acid in rats using <sup>1</sup>H NMR-based metabolomic approach.

Rat urinary metabolome were examined to study time-course and dose-response of maleic acid. Adult male SD rats were divided into control, low-dose (6 mg/kg), medium-dose (20 mg/kg), and high-dose (60 mg/kg) and treated with vehicle or maleic acid via oral gavage daily. Urine samples collected on day and night at 1, 7, 14, 21, and 27 days were examined by high-resolution <sup>1</sup>H nuclear magnetic resonance (NMR) followed by multivariate statistical analysis. The principle component analysis (PCA) score plots from the analysis of urinary metabolome showed changes of metabolome patterns within different exposure groups. Clear metabolome separation between high-dose and the control groups were observed from the night samples of day 14 and later. The increased levels of acetoacetate and hippurate, and decreased levels of alanine and acetate in the treatment groups were observed in the night samples of day 27. Changes of metabolites are related with environment stress and energy metabolism. More metabolites contributing to the grouping along dose- or time- series will be identified. By investigating the perturbation of urinary metabolome in the rats can assist urinary biomarker discovery for maleic acid and find out possible toxic mechanisms induced by maleic

**Poster #: 39****Abstract #: 2086**

**Abstract Title:** Applying an untargeted Metabolomics workflow linking HRAM QTOF data to biology enabled to increase arginine production in *C. glutamicum* by rational strain design

**Authors:** Michael Gerlich, Frederik Walter, Marcus Persicke, Aiko Barsch, Heiko Neuweiger, Matthias Szesny, Nikolas Kessler, Klaus Meyer, Jörn Kalinowski,

**Presenting Author Affiliation:** Bruker Daltonik GmbH

**Abstract Submission:**

*Corynebacterium glutamicum* is a biotechnological workhorse for the production of amino acids and other primary metabolites. Arginine is a glutamate-derived amino acid that is used in the cosmetic and pharmaceutical industries and as a food additive.

The genes for biosynthesis of arginine are organized in an operon structure (*argCJBDFRGH*). The suboperon *argGH* is transcribed from an additional promoter. The genes are regulated by the repressor *argR*, which is activated by arginine. Additionally, the N-acetylglutamate kinase encoded by *argB* is feedback-regulated by arginine. Ikeda et al. [1] reported the rational construction of arginine-producing *C. glutamicum* strains by chromosomal deletion of *argR* and introduction of feedback-resistant *argB* alleles. Besides arginine, these strains accumulated significant amounts of citrulline as a by-product, indicating a bottleneck in the pathway.

Here we investigate three *C. glutamicum* arginine biosynthesis mutant strains by non-targeted HRAM LC-QTOF based metabolomics. The novel MetaboScape software was used for data processing and interpretation. A customized analyte target list enabled automatic annotation of the extracted features according to user-definable confidence levels for retention time, accurate mass, and isotopic pattern information. An unknown compound — more abundant in the mutant strains — could tentatively be identified as glutamylvaline by molecular formula generation and database query. Mapping changes of the identified compounds on biochemical pathway maps enabled quick formulation of hypotheses for the observed changes in the biological context.

These presented results demonstrate that combining rational strain design with non-targeted metabolomics is a powerful tool to increase production of desired metabolites in biotechnological workhorses.

[1] Ikeda M., et al. Appl Environ Microb 2009, (75): 1635–1641.

**Poster #: 40****Abstract #: 2301****Abstract Title:** MetExplore: handling genome scale metabolic networks online**Authors:** Florence Vinson, Ludovic Cottret, Yoann Gloaguen, Benjamin Merlet, Florence Maurier, Floréal Cabanettes, Maxime Chazalviel, Sanu Shameer, Clément Frainay, Nathalie Poupin, Fabien Jourdan,**Presenting Author Affiliation:** INRA**Abstract Submission:**

Genome scale metabolic networks provide an enlightening context for the analysis of metabolic profiles since they gather information about reactions that consume or produce metabolites. This integrative view of metabolism nevertheless implies dealing with networks containing thousands of densely connected reactions (7,440 for human metabolic network). To make sense of these large networks, they need to be turned into mathematical objects called graphs (nodes connected by edges) from which dedicated algorithms can be used to identify less dense and more informative sub-networks. Once selected, visualization tools can allow in-depth analysis of these sub-networks.

Since 2009 the MetExplore web server ([www.metexplore.fr](http://www.metexplore.fr)) has offered access to more than 250 published networks representing a large variety of organisms. A key feature of MetExplore is its provision of a single framework that allows analysis of omics datasets within the context of these networks. MetExplore allows:

- Interactive browsing of metabolic network contents
- Mapping omics data
- Pathway enrichment
- Fully online and interactive visualization
- Constraint based modelling for flux analysis
- Large range of webservice
- Collaborative metabolic network curation

MetExplore also enables users to create or modify their own networks. Private metabolic networks can be created from scratch or previously created personal models can be uploaded to MetExplore as SBML or tabulated files and then further modified using the curation tool. The same analysis and visualization tools that are available for public networks can then be applied.

MetExplore is offered as a freely accessible service to the community. It has been successfully used to interpret metabolomics data obtained from a range of research areas including microbiology, pharmacology, food toxicology and parasitology.



MetExplore is an INRA project in collaboration with the University of Glasgow. MetExplore is mainly funded by French National Infrastructure for Metabolomics and Fluxomics (MetaboHub) and EU project PhenoMeNal (EC-654241).

**Poster #: 41**

**Abstract #: 2501**

**Abstract Title:** Model-free analysis of NMR and MS spectra for metabolic tracer experiments

**Authors:** Mei Chong, Anusha Jayaraman, Silvia Marin, Vitaly Selivanov, Pedro de Atauri Carulla, Daniel Tennant, Marta Cascante Serratos, Ulrich Günther, Christian Ludwig,

**Presenting Author Affiliation:** Kings College London

**Abstract Submission:**

Mammalian cells are multi compartment entities with different isoforms of the same enzyme often present in different parts of the cell. This compartmentalization of metabolism complicates the interpretation of stable isotopes tracer studies, which are usually performed on whole cell extracts and rely on simulations using pre-defined models of metabolism. Here we present a new algorithm whereby a simultaneous analysis of NMR and MS data overcomes the necessity for a priori metabolic modelling and yields a model-free isotopomer distribution thus allowing a better understanding of metabolic mechanisms. We demonstrate that NMR multiplets and MS isotopologues can be consistently fitted to isotopomer/isotopologue distributions for key metabolites in central metabolism from a single set of samples.

**Poster #: 42**

**Abstract #: 2131**

**Abstract Title:** Deriving strategies of metabolic network regulation from metabolomic time series data

**Authors:** Thomas Naegele, Lisa Fuertauer, Matthias Nagler, Jakob Weizmann, Wolfram Weckwerth,

**Presenting Author Affiliation:** Dept. Ecogenomics and Systems Biology, University

**Abstract Submission:**

Connecting experimental metabolic time series data with biochemical network information is a central issue in systems biology. Experimental analysis of diurnal, circadian or developmental dynamics of metabolism frequently results in a comprehensive and multidimensional data matrix comprising information about metabolite concentrations, protein levels and/or enzyme activities. While transcriptomics, proteomics and metabolomics experiments are part of many systems biology studies, functional data integration in a biochemical and physiological context is still challenging. Here, a computational approach is presented addressing the functional connection of experimental time series data with biochemical network information. Based on a time-continuous and variance-weighted regression analysis of experimental data, metabolic functions, i.e. first-order derivatives of metabolite concentrations, are related to time-dependent changes in other biochemically relevant metabolic functions. This unravels time points of perturbed dependencies in metabolic functions indicating a modified biochemical interaction. The approach is validated using previously published experimental data on a stress-induced time course of primary and secondary metabolite levels. To support and ease the computational application of the presented approach of functional time series analysis, a graphical user interface was developed which will briefly be introduced.

**Relevant Literature:**

Nägele T, Fürtauer L, Nagler M, Weizmann J, Weckwerth W (2016) A Strategy for Functional Interpretation of Metabolomic Time Series Data in Context of Metabolic Network Information. *Frontiers in Molecular Biosciences* 3. doi:10.3389/fmolb.2016.00006

**Poster #: 43**

**Abstract #: 2392**

**Abstract Title:** Metabolomic Analysis of Plasma on Alternate Day Fasting in Metabolic Syndrome Patients by UHPLC-QTOF/MS

**Authors:** Mi-Ri GWON, Bo Kyung Kim, Boram Ohk, Ji-Won Lee, Sook Jin Seong, Young-Ran Yoon,

**Presenting Author Affiliation:** Kyungpook National University

**Abstract Submission:**

Alternate Day Fasting (ADF “feed day”, alternated with 25% energy intake “fast day”), is effective for weight loss, and prevention and improvement of 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 examine the differences of endogenous metabolites after ADF in metabolic syndrome patients.

Thirty-six subjects with metabolic syndrome (BMI 23.1-39.3 kg/m<sup>2</sup>) were randomized into four groups: ADF and exercise (aerobic and muscle exercise) only ADF only exercise group or 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 groups. Highly correlated candidate metabolites, according to variable importance projection (VIP) value > 1.0, were selected using a OPLS-DA model. Several metabolites explored were used to determine their importance for the metabolism in metabolic syndrome patients.

These findings suggest that ADF and exercise is the best effective for weight loss and prevention and improvement of overweight and metabolic syndrome, although further research implementing larger sample sizes and strict clinical trial design are required before solid conclusion can be reached. Also, our study represents the first UHPLC-QTOF/MS-based metabolomic study to evaluate the influence of ADF and exercise on the metabolite patterns in overweight and metabolic syndrome. This kind of UHPLC-QTOF/MS-based metabolomic study could serve as a useful tool to evaluate the effect of dietary and exercise control and identify the metabolic syndrome mechanisms.

**Poster #: 44**

**Abstract #: 2445**

**Abstract Title:** New bioinformatic tools for metabolomics

**Authors:** David Wishart, Yannick Djoumbou Feunang, Craig Knox, Tanvir Sajed, Felicity Allen, Yifeng Liu, Zheng Shi, Zachary Budinski, Russell Greiner,

**Presenting Author Affiliation:** The Metabolomics Innovation Centre

**Abstract Submission:**

Metabolomics faces a number of challenges. First, more than 200,000 natural products or metabolites are known – but information about their origins, purposes, biological or chemical roles, structural features and other properties are only available for 2 million feasible structures along with the corresponding predicted MS/MS and EI-MS spectra and then making these publicly available, we believe this could go a long way towards solving the “unknown-unknown” problem in metabolomics.

**Poster #: 45**

**Abstract #: 2602**

**Abstract Title:** IROA Fluxomic analyses: a non-targeted, all-encompassing protocol using IROA (Isotopic Ratio Outlier Analysis)

**Authors:** Chris Beecher, Felice de Jong, Tim Garrett, Rick Yost,

**Presenting Author Affiliation:** UF, SECIM

**Abstract Submission:**

All IROA protocols incorporate stable-isotopes into metabolites, creating unique isotopic patterns in all metabolites. The IROA Fluxomic protocol is used to first label every metabolic pool with a 5%  $^{13}\text{C}$  isotopic pattern, and then introduces a specific precursor whose flux is to be determined as a 95% to 99%  $^{13}\text{C}$ -labeled compound. Since every metabolic pool is labeled the IROA ClusterFinder software can easily identify all metabolic pools, differentiate them from artifacts and noise, and since their exact pattern is known, automatically seek any perturbations in the expected  $^{13}\text{C}$  isotopic pattern that would indicate flux into that metabolic pool. Unlike other fluxomic approaches this process is completely automated, and examines every metabolic pool without the bias or the need to predetermine it for investigation.

We will present a series of experiments in which the flux of glucose and glutamine are separately examined as the flux agent, in a HepG2 cell in a time-course experimental system. Our results will be compared with similar experiments done using a more traditional approach. In the traditional approach, specific metabolic pools are queried and the label may be targeted to obtain highly specific information often detailing which carbon is transferred (i.e. positionally). In the IROA Fluxomic approach, the total number of carbons derived from the flux agent and transferred into every metabolic pool is easily determined however positional effects are not available. These two techniques are therefore very complimentary, and increase the ability to understand flux as a function of physiological change.

The IROA ClusterFinder software was specifically modified to complete the entire unbiased analysis with no preconceptions. It affords a new and unique fluxomic point of view.

**Poster #: 46**

**Abstract #: 2364**

**Abstract Title:** A combined multi-omics and in silico approach to decipher metabolic network shifts during the differentiation of human hepatocyte

**Authors:** Nathalie Poupin, Anne Corlu, Nicolas Cabaton, Hélène Dubois-Pot-Schneider, Cécile Canlet, Clément Frainay, Florence Vinson, Marie-Anne Robin, Daniel Zalko, Fabien Jourdan,

**Presenting Author Affiliation:** UMR1331, TOXALIM (Research Center in Food Toxicology)

**Abstract Submission:**

The hepatic cell line HepaRG, which is increasingly used in toxicity studies, has the particularity to differentiate from progenitor to mature hepatocyte-like cells. In order to explore the metabolic shifts occurring during this differentiation process, we identified the functional metabolic network of HepaRG cells at each developmental stages: day 3 (progenitors) and day 30 (differentiated cells). Gene expression and metabolomic (1H NMR) data obtained from HepaRG cells at the two stages were integrated in the context of the global human genome-scale metabolic network Recon2. We used a modified version of the iMAT algorithm developed by Shlomi et al. to identify, based on these data, the sub-networks of reactions specifically active in HepaRG cells at each developmental stage. For each stage, we identified several sub-networks of active reactions, having an equivalent adequacy to experimental data. We applied classification analysis methods to explore intra- and inter-stages variability among these sub-networks. We showed that, for each stage, the heterogeneity between sub-networks was mainly caused by the occurrence of several alternative reactions or the relative low contribution of transcriptomic data in some pathways. To better characterize the systemic metabolic capacities of the cells, we chose, contrary to most approaches, to consider the whole set of similarly adequate sub-networks, since it allows taking into account various metabolic alternatives. Through simulations and pathway enrichment analyses, we predicted that differentiated cells would globally be able to perform a larger number of liver-specific functions (e.g., urea production) and we identified several sets of reactions that were differently active between the two stages. These reactions mostly belong to pathways specific to hepatic activity (e.g., bile acid synthesis) but also to fatty acid synthesis and oxidation pathways. About 50% of the predicted modulated reactions were not evidenced from transcriptomic data and were « newly » inferred by the computational models.

**Poster #: 47**

**Abstract #: 2307**

**Abstract Title:** Visualization of metabolic networks and pathways with MetExploreViz

**Authors:** Maxime Chazalviel, Florence Vinson, Clement Frainay, Benjamin Merlet, Yoann Gloaguen, Floréal Cabanettes, Ludovic Cottret, Nathalie Poupin, Fabien Jourdan,

**Presenting Author Affiliation:** INRA - ToxAlim

**Abstract Submission:**

The metabolic network of an organism gathers all the biochemical reactions that can occur in this organism, regardless of the conditions. In order to visualize those networks we introduce MetExploreViz, a free and open source javascript library which can easily be embedded into a webpage. One of the aims of MetExploreViz is to allow the extraction of sub-networks of interest based on a list of metabolites obtained from metabolomics experiments. To do so, a lightest-path based algorithm is used to identify the sub-networks linking the metabolites of interest and to highlight them in the network representation.

Within MetExploreViz, imported omics data can also be pinpointed on the network representation using mapping functions. Moreover, many features have been implemented in order to facilitate visual mining of the network. Especially, MetExploreViz offers a dynamic display of the network (contrary to the static traditional maps), allowing users to move nodes and automatically rearrange the layout to avoid node overlappings. Users can also choose to hide over-linked side compounds (e.g. H<sub>2</sub>O, H<sup>+</sup> ...) in order to reduce the amount of edges in the network and facilitate the visualization. Cellular compartments can be highlighted using color schemes, and a contextual menu allows displaying information from MetExplore database on compounds or reactions. MetExploreViz also enables users to save their network drawings and sessions in order to resume their work. Finally, publication ready images can be exported in various formats (jpeg, png or svg).

MetExploreViz will be integrated in the e-infrastructure developed in the framework of PhenoMeNal project (EC-654241).



**Poster #: 48**

**Abstract #: 2318**

**Abstract Title:** Inferring missing compounds from metabolic profile using network topology analysis

**Authors:** Clément Frainay, Nicolas Weiss, Benoit Colsch, Frédéric Sedel, Dominique Thabut, Christophe Junot, Fabien Jourdan,

**Presenting Author Affiliation:** Toxalim, Université de Toulouse, INRA, Toulouse

**Abstract Submission:**

Monitoring and understanding the metabolic status of an organism under various environmental or genetic conditions is a key challenge in human health or bioengineering. Untargeted metabolomics, combined with multivariate statistics, allow detecting metabolites with significant changes in concentration. The resulting list is a metabolic profile that characterizes the physiological response of the organism to the perturbation being studied. Those profiles allow to compare perturbations and to understand the underlying biological mechanism using pathway enrichment, path search among genome-scale metabolic network or in silico flux analysis. However, no single metabolomics technology enables all metabolites to be monitored and metabolomic experiments driven from biofluid sample overshadow non-excreted compounds. Thus, metabolic profiles remain incomplete.

Taking advantage of the increasing availability of genome-scale metabolic network gathering all the biochemical reaction for a given organism, we propose a new bioinformatics method to infer potential missing compounds from a metabolic profile, based on the identification of nodes of influence in a metabolic network. This kind of analysis is derived from the Google's PageRank computation, allowing ranking nodes given their influence on a specified set of nodes of interest, which was successfully applied on social network and World Wide Web analysis. Several adaptations have been led to take into account the complex nature of relationship between compounds in a metabolic network, in order to ensure biologically meaningful results. The method was applied to metabolic profiles identified in cerebrospinal fluid from patients with hepatic encephalopathy and raised some unidentified compounds that could be linked to the syndrome according to the literature. This method will soon be implemented in the MetExplore web server and will be applicable to any user-defined network and does not require advanced settings, as purely topology-based.

**Poster #: 49**

**Abstract #: 2502**

**Abstract Title:** Metabolic Pathways and Networks Integration with MetaboLights: Visualisation and Analysis

**Authors:** Venkata Nainala, Benjamin Merlet, Florence Vinson, Maxime Chazalviel, Fabien Jourdan, Egon Willighagen, Reza Salek, Kenneth Haug, Christoph Steinbeck,

**Presenting Author Affiliation:** EMBL-EBI

**Abstract Submission:**

Since its official launch in summer 2012, EMBL-EBI has established the MetaboLights database as the most successfully open metabolomics repository internationally. MetaboLights is growing very rapidly and currently host MS and NMR experiments, complete with raw and processed data. Presently, MetaboLights has about 300 complete metabolomics experiments, 23000 reference metabolites and about 2100 different organisms reported from studies and reference compounds. MetaboLights is endorsed by journals and fully supports secure private access to restricted or pre-publication datasets.

Current techniques for metabolomic analysis aim at identifying known metabolites already mapped to reference metabolic networks and pathways. MetaboLights studies normally have a large number of both known and unknown metabolites reported. Projecting these available metabolic profiles onto biochemical network and pathway knowledge bases is invaluable for the interpretation of biological studies. The MetaboLights team are investing a large effort into integrating available open source pathway tools directly onto submitted primary experimental data, bringing analysis-to-the-data.

We have already integrated some commonly used open platforms like WikiPathways and MetExplore, both are dedicated to the curation and visualisation of the biological pathways. These tools facilitates the integration of metabolomics fully into the rest of the bioinformatics analysis pipeline, supporting hypotheses for underlying disease mechanisms of action and pinpointing the mechanisms for individual differences in cellular phenotypes.

Integration of free and open comprehensive pathway analysis tools greatly enhances the utility of MetaboLights for the metabolomics community.

This integration is part of the e-infrastructure developed in the framework of PhenoMeNal project (EC-654241). The development of MetaboLights is funded by the BBSRC, grant references BB/L024152/1 & BB/I000933/1.

**Poster #: 50**

**Abstract #: 2600**

**Abstract Title:** Imaging of Muscle and Adipose Tissue by MALDI-MS for Exercise Metabolomics

**Authors:** Michelle Reid, Irwin Kurland, Yunping Qiu, Timothy Garrett, Richard Yost,

**Presenting Author Affiliation:** University of Florida

**Abstract Submission:**

Glucose (Glc) incorporation in muscle and adipose tissue is enhanced through exercise regimes however, the direct mechanism and metabolic interactions are poorly understood. Our hypothesis is that metabolic network(s) of exercise response, in tissues and biomarkers in plasma can be developed using a U 13C-labeled glucose tolerance test (GTT).

All mouse groups were given 2 mg/gm body weight intraperitoneal (ip) glucose (U-13C labeled-Glc or unlabeled-Glc, for background subtraction) 10 minutes prior to exercise. The rest (R) group was injected with unlabeled-Glc and then rested for 35 mins. The exercise to exhaustion groups (EX) ran on a treadmill for 30 mins and one group received labeled-Glc, the other unlabeled-Glc. The EX plus rest (EX+R) group ran for 30 mins rested for 35 mins, and one group received labeled-Glc, the other unlabeled-Glc. All mice were sacrificed immediately upon completion of treatment, and tissues rapidly frozen in liquid nitrogen then stored at -80°C. When ready for sectioning, the samples were quickly placed in a -35°C cryostat microtome and thaw mounted onto a glass slide. A Meinhard nebulizer coated the samples with 9-AA and DHB matrices, for negative and positive modes, respectively. Imaging data were collected with a MALDI Thermo Scientific LTQ XL mass spectrometer.

Preliminary muscle data indicate that LPC (18:0) and LPC (22:6) decrease during exercise when compared to resting state. In addition, the adipose tissue shows a decline in PC (18:1/18:1). Plasma LPC 18:2 M3/M0/glycerol M3/M0 was between 6-8%, for mice given ip U-13C Glc, with EX (8%) or Ex+R (6%), suggesting dynamic rearrangements of glucose metabolic flux towards the glycerol backbone of specific lipid biomarkers, as lipogenesis is inhibited with exercise. Current studies are evaluating U-13C incorporation from glucose to acylcarnitines, to further identify significant metabolites that are markers of exercise metabolism.

**Poster #: 51**

**Abstract #: 2223**

**Abstract Title:** Plant Metabolomics at Bordeaux Metabolome Facility, a member of MetaboHUB and PHENOME IA projects. Tools and Applications

**Authors:** Catherine Deborde, Patricia Ballias, Camille Bénard, Stéphane Bernillon, Thierry Berton, Cécile Cabasson, Virginie Cocureau, Salimata Diarrassouba, Yves Gibon, Daniel Jacob, Marie Lefebvre, Mickaël Maucourt, Dominique Rolin, Simo

**Presenting Author Affiliation:** INRA PMB-MetaboHUB

**Abstract Submission:**

The Bordeaux Metabolome Facility develops plant metabolomics and high-throughput metabolic phenotyping:

- 1- Quantitative metabolic profiling of plant organs or tissues by <sup>1</sup>H-NMR [1,2,3] and Fast 2D NMR[4]. On-going developments by LC-MS.
- 2- Plant metabolomics by LC-HRMS [2,3,5] or lipidomics by LC-HRMS [6,7] and by GC-MS [8],
- 3- Robotised high-throughput measurements of metabolite concentrations and enzyme activities and kinetics[3,9,10],
- 4- Storage of metadata and raw data and biostatistical analysis through web-based applications: “MeRy-B” (<http://bit.ly/meryb>) and BioStatFlow (<http://biostatflow.org>) [11],
- 5- Development of spectra processing methods for metabolite identification [12] and open source software for processing and visualization of <sup>1</sup>H-NMR metabolomics data (<http://nmrprocflow.org>),
- 6- Identification of metabolic markers for biotic or abiotic environmental changes [3,13], plant-pathogen interactions [15], or plant performance,
- 7- Modelling of fruit metabolism [16,17],
- 8- Integration of metabolomics data with other ‘omics data for the study of fleshy fruit development and metabolism [18].

This poster will provide an overview of some major features of metabolomics studies and tools developed at Bordeaux and show our commitment to develop solutions that will help creating knowledge rather than just data.

Acknowledgement: MetaboHUB (ANR-11-INBS-0010) and PHENOME (ANR-11-INBS-0012) projects.

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**Poster #: 52**

**Abstract #: 2297**

**Abstract Title:** Cell Culture Media: NMR Approaches to Evaluating Quality and Nutrient Consumption

**Authors:** Kimberly Colson, K. Brian Killday, Christian Fischer,

**Presenting Author Affiliation:** Bruker BioSpin

**Abstract Submission:**

Cell culture media is widely used to provide nutrients for cell growth in research, diagnostic and manufacturing applications. Use in research and manufacturing applications to produce secondary metabolites from microbes is long standing. Cell culture media use is increasing as a result of the growing focus on the development and production of biologics and biosimilars in the pharmaceutical industry. Typical cell culture media contain a mixture of defined nutrients that are expected to produce the desired product. The nutrients are often small molecule components that may be monitored using NMR spectroscopy utilizing a metabolomics approach.

In this poster we present techniques that we are developing to enhance the analysis of cell culture media. The ability for automated NMR analysis for quality control and monitoring post cell addition was evaluated. Solvent suppression and region selective 1D and 2D homonuclear and heteronuclear NMR techniques enhanced the monitoring of nutrients consumed and characterization of metabolites produced in the media. Drift compensation features of modern NMR systems provided the ability to obtain spectra of the pure media without requiring addition of a deuterium lock solvent, allowing for real time NMR monitoring of the media. These studies evaluate the ability to monitor fermentations in either (1) batch mode, sampling aliquots at time points and submitting them for analysis, or (2) continuous monitoring in flow mode (reaction monitor). NMR monitoring may allow optimization of real time conditions of the fermentation for maximum production of a desired product.

**Poster #: 53**

**Abstract #: 2009**

**Abstract Title:** Fully automated structure verification of organic compounds by combining accurate MS with NMR data analysis

**Authors:** Wiebke Timm, Jens Decker, Sven Brehmer, Sandra Groscurth, Peter-Réne Steiner, Till Kühn, Jens Fuchser,

**Presenting Author Affiliation:**

**Abstract Submission:**

The structures of tentatively identified biomarkers can be verified in targeted experiments. LCMS and NMR can be used complementarily for this task. Here we describe a new approach which automates structure verification and is easy to use by non-experts. Verification results are visualized with a red/yellow/green classification. The scientist obtains a combined classification for NMR and MS data. Further visual inspection of the data is possible but not mandatory.

The MS analysis workflow includes automatic re-calibration, extracted ion chromatogram calculation for target compounds, saturation masking, and the validation of accurate mass data and isotopic distributions. A robust evaluation of the statistics over individual mass spectra is part of the novel isotope pattern validation procedure. The compositional nature of the isotopic data is taken into account [Aitchison, 1982]. The sensitivity/specificity-tradeoff and rejection criteria for the classification are configurable. NMR spectral analysis yields fully assigned spectra based upon the quality of fit and the similarity between predicted and actual spectral parameters.

96 commercially available standards [Specs, Netherlands] were measured in 2 different labs (LCMS and NMR, respectively). For LCMS a UHPLC (RSLC, Dionex) was coupled to an ESI-Q-TOF (compact, Bruker Daltonics). To each analysis an external calibrant was added via a switching valve. For NMR data 1H and HSQC spectra were recorded using a 400 MHz NMR spectrometer (AVIIIHD 400MHz, Bruker BioSpin).

Validation of the MS data processing result using five isobaric decoy formulas with similar elements for each of the 96 samples resulted in 1% FPR at 89.6% TPR using a medium-conservative sensitivity/specificity-tradeoff. The molecular formula is classified by MS, but not the structure.

For the NMR, another dataset with decoys with similar molecular structure has been created to pose a similarly difficult classification task. That results in a 1.6% FPR at 96% TPR with respect to the correct structure.

**Poster #: 54**

**Abstract #: 2060**

**Abstract Title:** Comprehensive profiling of bile acids in human and mouse

**Authors:** Jun Han, Georgia Mitsa, Lin Karen, Christoph Borchers,

**Presenting Author Affiliation:** UVic-Genome British Columbia Proteomics Centre

**Abstract Submission:**

Bile acids are steroidal end-products that are synthesized from cholesterol in hepatocytes. They play key roles in human physiology but also have a wide range of cytotoxicity which is associated with pathology. To comprehensively characterize the bile acid (BA) profiles of various human and mouse biological samples, a dual (pH and organic solvent)-gradient reversed-phase UPLC-multiple reaction monitoring (MRM)/MS method was developed to simultaneously separate 69 known BA standards. The ion transitions for each isomeric BA group were then used to construct class-specific MRM lists for untargeted detection of aminated and esterified BA conjugates. A chemical derivatization - UPLC/MRM-MS approach with 3-nitrophenylhydrazine as the derivatizing reagent was also developed for the analysis of unconjugated BAs in a similar manner.

Class-specific UPLC/MRM-MS resulted in the successful detection of >40 glycine- and taurine-conjugated BAs with unknown structures in mouse blood and liver, and >30 of these species in human blood, urine, and feces. The assignments were confirmed by UPLC-ultrahigh-resolution MS, collision-induced MS2, and neutral loss- and precursor ion-dependent MS3. Chemical derivatization-UPLC-MS/MS enabled the detection of at least 15 putative unconjugated mono-/di-/tri-OH BAs and nor-BAs in the tested samples. Many of the detected BA sulfates and glucuronides in human urine were not among the known BA conjugates. The major BAs detected in human feces were secondary BAs and their oxo-, keto- and dehydroxylated BA metabolites with known or unknown structures, resulting from co-metabolism regulated by gut microbiota. To identify the putative BA glucuronides, UPLC fractionation,  $\beta$ -glucuronidase deconjugation, and re-analysis of the deconjugated fractions by UPLC/MRM-MS were performed. The authentic compounds of various BA sulfates were synthesized and used to identify the putative BA sulfates. Based on these analyses, more than 140 BAs were profiled in the human and mouse samples, many of which were quantified for the first time.



**Poster #: 55**

**Abstract #: 2174**

**Abstract Title:** The development of compound annotation technique extended for conjugates.

**Authors:** Tairo Ogura, Takeshi Bamba, Akihiro Tai, Eiichiro Fukusaki,

**Presenting Author Affiliation:** Shimadzu Scientific Instruments, Inc.

**Abstract Submission:**

Metabolites of bioactive compounds are often found in a biological samples as a conjugated form. Liquid chromatography coupled to high-resolution and accurate mass spectrometry and multistage collision-induced dissociation (CID) can provide product ion spectra including structural information of each metabolites. The current database such as PubChem and ChemSpider become huge and are still increasing, however the compound annotation of conjugated compound is still difficult. Therefore, we developed a novel technique to annotate compounds, including conjugates, using the combination of in-silico conjugation and compound databases. The annotation of conjugates is conducted in three steps: recognition of the type and number of conjugates, compound annotation of the deconjugated form, and In-silico evaluation of the conjugated candidate. To evaluate the performance of basic compound annotation technique including spectrum assignment and scoring, we compared the results of compound annotation against previously reported techniques. To evaluate the performance of developed approach for conjugates, we conducted compound annotation for product ion spectrum of commercially available compound and mouse urinary conjugates. As a result of compound annotation, the glucuronide and sulfate conjugates of catechin and its C-ring fusion metabolite were shown as candidates despite these conjugates were not listed in Compound database. These results show that the developed approach can annotate compound including sulfate and glucuronide conjugate successfully. In this study, we focused on glucuronide and sulfate as important urinary conjugates. However, there are other reactions worth investigating, such as glutathione conjugation, glycine conjugation, methylation, and acetylation. This approach can be applied to these conjugates in the same way, because it is known that these conjugate reactions also generate specific neutral loss.

**Poster #: 56**

**Abstract #: 2070**

**Abstract Title:** Monitoring Glutathione Redox Reaction in Living Human Cells by Heteronuclear NMR and Its Application to Anticancer Drug Resistance

**Authors:** Soeun Kang,

**Presenting Author Affiliation:** Seoul National University

**Abstract Submission:**

Glutathione (GSH) redox reaction is critical in the defense against cellular reactive oxygen species (ROS). However, direct and real-time monitoring of such reaction in living mammalian cells has been hindered by the lack of a facile method. Herein, we describe a new approach exploiting the GSH biosynthetic pathway and heteronuclear NMR. <sup>13</sup>C-labeled cysteine was incorporated into GSH in U87 glioblastoma cells, and the oxidation of GSH to GSSG by an ROS-producing agent could be monitored in living cells. Further application of the approach to cells resistant to temozolomide (TMZ), an anti-glioblastoma drug, suggested a possible new resistance mechanism involving neutralization of ROS. The result was also corroborated by observing the up-regulation of glutathione peroxidase 3 (GPx3). This new approach can be easily applied to redox-dependent signaling pathways and drug resistance involving ROS.

**Poster #: 57**

**Abstract #: 2215**

**Abstract Title:** Targeted and untargeted strategies for analysis of dynamic labeling experiments using LC-HRMS

**Authors:** Johannes Hartl, Patrick Kiefer, Julia Vorholt,

**Presenting Author Affiliation:** ETH Zürich

**Abstract Submission:**

Liquid-chromatography coupled to mass spectrometry is a key technology in metabolomics. However, metabolite analysis by LC-MS is generally hampered by chemical noise resulting in a majority of peaks that is not related to metabolites. Introducing isotopically labeled metabolites into the sample by feeding labeled substrates to the cells of interest allows for efficient filtering of isotopic patterns resulting from incorporation of labeled atoms into the metabolite. The isotope dilution method adopts this principle and is widely used to identify and quantify metabolites. Labeled substrates can also be used to perform dynamic labeling experiments providing crucial information on the dynamics of metabolites in biological systems, and in turn the activity of involved reactions. In fact, they have been used to elucidate pathways for a long time. While labeling experiments have many advantages, they also pose challenges, including increased complexity in the chemical space of the sample, and the necessity to analyze and relate multiple samples to one time course with isotopologue patterns changing over time when analyzing labeling dynamics. Using LC-HRMS data we introduce different strategies for the analysis of (dynamic) labeling experiments using stable isotopes. Employed pipelines for data analysis are built on the python based open source framework eMZed2. Based on examples with varying model organisms, we outline automated targeted as well as untargeted approaches for analysis of dynamic LC-HRMS data of level one and two. Additionally, we developed an extensive tandem HRMS approach to obtain positional dynamic labeling information. In summary, we introduce workflows for facilitated analysis of dynamic labeling experiments and highlight potential applications in metabolomics studies.

**Poster #: 58**

**Abstract #: 2276**

**Abstract Title:** New Strategies for Identification of Metabolites in NMR Spectra using Databases, e.g. HMDB

**Authors:** Christian Fischer, Kimberly Colson,

**Presenting Author Affiliation:** Bruker BioSpin GmbH

**Abstract Submission:**

Identification and assignments of metabolites is an important step in metabolomics. It is necessary for quantification and the discovery of new biomarkers.

In general there are two use cases for identification of compounds:

1. The constituents of the sample are unknown.
2. The constituents of the sample are known. Identify if a target compound appears / disappears.

Conventional approach involves a database search, wherein chemical shifts are assigned to specific metabolites by use of a tolerance limit. This approach uses only a fraction of the information that is readily available in a metabolomics study. Utilization of other information such as biological information, additional NMR characteristics, and signal/metabolite identifiability can be used to improve confidence of the identification. The extension of utilized information enhances metabolite identification in both use cases.

Presented here is the development of an automated identification strategy for use case 2 that guides metabolomics researchers in identification of key components.

**Poster #: 59**

**Abstract #: 2188**

**Abstract Title:** Acylcarnitine profiling in plasma and tissues of NZO mice as model for obesity-induced T2 diabetes

**Authors:** Anna Weiser, Pieter Giesbertz, Hannelore Daniel, Britta Spanier,

**Presenting Author Affiliation:** TU München

**Abstract Submission:**

Metabolomics has identified biomarkers in human plasma with a predictive quality for type 2 diabetes (T2D) development. Amongst these markers are various acylcarnitine species, most prominently those derived from branched-chain amino acid (BCAA) breakdown. The metabolic perturbations in tissues underlying these changes in plasma are often unknown and hard to assess in humans. Thus animal models are used to have access to the different tissues. In contrast to monogenetic mouse models of obesity and diabetes, the New Zealand Obese (NZO) mouse is a prototypical polygenic model for obesity, with a male-specific susceptibility to developing diabetes. We have used this model to study the metabolic alterations in obesity-induced diabetes for changes in acylcarnitines.

12 male and 11 female NZO mice at an age of 8 weeks were fed a chemically-defined high carbohydrate diet for 12 weeks. Acylcarnitine profiles were obtained from plasma, skeletal muscle, liver and kidney tissues, using a high-resolution LC-MS/MS method. Around 45 acylcarnitine species originating from fatty acids and amino acids, as well as odd-numbered and dicarboxylic acylcarnitines are covered. Furthermore blood glucose, plasma insulin, non-esterified fatty acids (NEFA), triglyceride and urea concentrations were measured. Principal component analysis of plasma samples revealed a grouping of normoglycemic females, normoglycemic males and hyperglycemic males and this originated mainly from differences in concentrations of acylcarnitines derived from BCAAs. Hyperglycemic males were characterized by high insulin levels and low levels of NEFAs. In addition, large differences in the concentrations of long-chain acylcarnitines between male and female mice were observed. Amongst tissues and plasma, muscle showed the most similar acylcarnitine profile to plasma, whereas liver and kidney differed the most from plasma.

In conclusion, the alterations in acylcarnitine profiles in NZO mice largely overlap with findings in humans and this indicates the suitability of this model to study metabolic changes underlying human T2D.

**Poster #: 60**

**Abstract #: 2407**

**Abstract Title:** Metabolomic analysis of Fusarium head blight on wheat – elucidation of fungal attack

**Authors:** Alexandra Simader, Alexandra Parich, Manuel Hofer, Michael Sulyok, Maria Doppler, Christoph Bueschl, Barbara Steiner, Marc Lemmens, Hermann Buerstmayr, Gerhard Adam, Justyna Rechthaler, Rudolf Krska, Rainer Schuhmacher,

**Presenting Author Affiliation:** BOKU University Vienna, IFA-Tulln, AZ

### **Abstract Submission:**

#### Introduction

The plant pathogen *Fusarium graminearum* (Fg) is able to infect wheat plants thereby leading to severe yield and quality losses as well as contamination with mycotoxins. In order to develop knowledge-based strategies against Fusarium head blight (FHB) it is greatly desired to systematically investigate the interactions between Fg and wheat plants.

#### Methods

In a recently performed metabolomics experiment two near isogenic wheat lines differing in resistance QTLs *Fhb1* and *Qfhs.ifa-5A* were inoculated in the glass house with Fg with the aim to investigate changes in the metabolome over time. Resistant CM-NIL-38 and the susceptible wheat line CM-NIL-51 were harvested at 0, 3, 6, 12, 24, 36, 48, 72, 96 hours after treatment with Fg. Mock and no treatment served as control. The use of a <sup>13</sup>C-assisted untargeted LC-HRMS approach allowed to investigate the secondary metabolites, whereas additional targeted GC-MS analysis was applied to complement the data by primary metabolites. Moreover, Fg reads from the transcriptomic analysis of the same data set served as reference for the fungal biomass. In order to discriminate between fungal attack and defense response of the host plant, metabolomics time course data such as Fg secondary metabolites as well as putative fungal biomarker compounds (e.g. mannitol, arabitol, xylitol, threitol, chitin, stachydrin) were investigated.

#### Results

Fg secondary metabolites and putative fungal biomarkers were detected as early as 24-36 hours after inoculation (hai). Time course of their formation has been investigated in detail and revealed mostly higher abundances of Fg secondary metabolites in the susceptible genotype at later time points, whereas at earlier time points some fungal metabolites were more abundant in the resistant wheat line. Additionally some metabolites could be investigated solely in Fg-treated samples exhibiting similar time course in the resistant genotype compared to the susceptible wheat line.

**Poster #: 61**

**Abstract #: 2412**

**Abstract Title:** New Insight into exudative Age-related Macular Degeneration (AMD): A Metabolomics Approach

**Authors:** Matthieu Schoumacher, Vincent Lambert, Sylvain Hansen, Justine Leenders, bernadette Govaerts, Bernard Pirotte, Jean-Marie Rakic, Agnes Noël, Pascal de Tullio,

**Presenting Author Affiliation:** Ulg (CIRM)

**Abstract Submission:**

Age-related macular degeneration (AMD) is a leading cause of vision loss in the western world in the elderly population. 90% of all vision loss due to AMD result from the exudative form, which is characterized by choroidal neovascularization (CNV). Age-related changes that induce pathologic CNV are incompletely understood. A successful application of anti-VEGF approaches in the clinic is obviously a turning point in AMD treatment. Nevertheless, despite such important advances, critical issues remain to be addressed. To better understand the etiology of this pathology, we decide to apply a <sup>1</sup>H NMR metabolomics approach on AMD patients and on a laser-induced murine choroidal neovascularization experimental model.

These experiments provide unique challenges to fulfill the goal of improving the current status of physiological information related to metabolome and in general to functional genomics.

For this purpose, sera from control and exudative AMD patients, induced and non-induced mice have been collected and the metabolic profiles of these samples were determined by <sup>1</sup>H NMR. After post-processing treatments, the different spectra were analyzed by statistical discriminant methodologies (PCA, ICA, PLS-DA, O-PLS-DA).

This approach allows the differentiation between control and AMD patients and between laser-induced mice and the control mice group. Moreover, the same discriminating spectral zones have been identified in human and mice model, leading to the emergence of different putative biomarkers. Among these markers, Lactate emerges as a key metabolite in both settings. Mechanistically, lactate produced locally and by inflammatory cells, plays a critical role in the onset of the inflammatory and angiogenic phases. In mice model of laser-induced CNV, normalization of circulating lactate by dichloroacetate, decreases CNV development. Our data support the innovative concept of lactate as a parainflammation- and angio-metabolite associated to AMD and CNV progression and metabolomics as a novel option for patients follow-up.

**Poster #: 62**

**Abstract #: 2166**

**Abstract Title:** Bioactive molecule discovery with large screening data

**Authors:** Tyler Backman, Thomas Girke,

**Presenting Author Affiliation:** University of California Riverside

**Abstract Submission:**

We demonstrate mining large publicly available data, to identify candidate bioactive small molecules with potential medical and biological utility, and candidate protein targets for known bioactive molecules and metabolites. This workflow is based on bioassayR, a software package we developed which systematically analyzes data from thousands of screening experiments to identify target selective small molecules and druggable protein targets. By simultaneously leveraging data from both custom small molecule screening efforts and public databases such as PubChem BioAssay and ChEMBL, bioassayR helps identify regions of the genome and proteome accessible to small molecule probes, elucidate novel mechanisms of action for bioactive molecules, and predict off-target effects which currently lead to a high attrition rate in drug discovery efforts. We also demonstrate how bioassayR integrates with a rapidly growing ensemble of R language cheminformatics tools curated by Bioconductor, including ChemmineR, a comprehensive cheminformatics environment also developed by our lab. bioassayR is an open-source R/Bioconductor package available from <https://bioconductor.org/packages/bioassayR/>.



**Poster #: 63**

**Abstract #: 2126**

**Abstract Title:** LC-MS analysis of raffinose family oligosaccharides in salt tolerant *Casuarina glauca* tissues

**Authors:** Tiago Jorge, Maria Florêncio, Ana Ribeiro-Barros, CARLA ANTONIO,

**Presenting Author Affiliation:** Plant Metabolomics Laboratory, ITQB NOVA

**Abstract Submission:**

Raffinose family oligosaccharides (RFOs) are non-structural, water-soluble carbohydrates widely distributed in the plant kingdom, and include the trisaccharide raffinose, the tetrasaccharide stachyose and the pentasaccharide verbascose. RFOs have been suggested to play a major role in storing carbohydrates in seeds and vegetative tissues, and in conferring osmoprotection against abiotic stresses, such as high salinity and drought.

The high polarity nature of RFOs makes their analysis with typical reversed phase liquid chromatography (RP-LC) generally difficult, with these compounds eluting very close to the void volume with minimal retention. In addition, these compounds lack an inherent chromophore in the ultraviolet-visible (UV) region to make them suitable for UV detection. To overcome these, alternative analytical methods based on LC coupled to mass spectrometry (MS) have been developed and porous graphitic carbon (PGC) stationary phases have been described in the literature to be a good alternative to typical RP columns for retaining these highly polar compounds. *Casuarina glauca* is a model actinorhizal plant native to the south east coast of Australia able to tolerate high levels of salinity therefore, it is commonly found in saline soils of the coastal zones and can be used to restore marginal soils and to prevent desertification.

The present work focus on the structural characterization of RFOs through collision induced dissociation (CID) and MS<sub>n</sub> experiments using a quadrupole ion trap mass spectrometer (QIT-MS) in the ESI positive ion mode. In addition, we describe the application of a PGC-ESI-QIT-MS<sub>n</sub> method for the quantitative analysis of RFOs in *Casuarina glauca* plant tissues under salt stress conditions.

**Poster #: 64**

**Abstract #: 2310**

**Abstract Title:** MULTIPLATFORM NON-TARGETED METABOLOMIC STUDY OF BRAIN TISSUE AT DIFFERENT POST MORTEM TIME POINTS

**Authors:** Carolina Gonzalez-Riaño, Silvia Tapia, Fernanda Rey-Stolle, Alberto Muñoz, Gonzalo Leon, Laura Ravanetti, Antonia Garcia, Javier de Felipe, Coral Barbas,

**Presenting Author Affiliation:** University CEU San Pablo

**Abstract Submission:**

Nowadays, neurodegenerative diseases have no clear biomarkers for their early diagnosis or treatment and metabolites are biomarkers of a broad range of central nervous system disorders serving as molecular drivers and by products of disease pathobiology. Their analysis in precisely dissected regions of brain is an appropriate strategy to follow. The only source of normal brain tissue is from autopsy but it is affected by changes due hypoxia, altered enzymatic reactions, etc. Thereby, metabolomics studies require attention to post mortem metabolism to determine if it affects metabolomics results.

Hippocampus is one of the first brain structures affected in age-related neurodegenerative diseases, such as Alzheimer's or Parkinson's diseases. A non-targeted metabolomic analysis was performed on hippocampus from left cerebral hemisphere of fifteen C57BL/6 mice, classified in three groups, differing in post mortem time: 30 min and 2 or 5 hours. For optimization of sample treatment, two extraction methods [1,2] were tested and best results were obtained using MeOH:H<sub>2</sub>O 50/50 (v/v) for homogenization and a combination of solvents MeOH:Methyl tert-butyl ether 320/80 (v/v/v) for extraction. With only 30 mg of tissue a multiplatform non-targeted metabolomic study based on UHPLC-ESI-QTOF-MS and GC-EI-Q-MS was performed. Data treatment along with univariate and multivariate statistical analysis were performed. Identification of metabolites was based on in house and commercial libraries.

When comparing findings from different post mortem time points, different metabolites including amino acids, biogenic amines, carbohydrates, lipids and nucleosides resulted altered due to cellular structures degradation, leading to cell lyses, and catabolic processes. Parallel neuroanatomical evaluation will be presented.

**Acknowledgments:** This work was supported by Spanish MINECO grant CTQ2014-55279-R. C. González-Riaño received a Fundación-Universitaria-San Pablo CEU grant.

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[2] V. Matyash, G. Liebisch, T.V. Kurzchalia, A. Shevchenko, D. Schwudke, J. lipid Res. 49(5) (2008) 1137-1146

**Poster #: 65**

**Abstract #: 2042**

**Abstract Title:** LC-MS-based metabolomics analysis of marine biofilm-forming bacteria: Impact of culture parameters and copper contamination

**Authors:** Laurie Favre, Annick Ortalo-Magné, Clément Coclet, Jean-François Briand, Jean-Charles Martin, Benjamin Misson, Cédric Garnier, Gérald Culioli,

**Presenting Author Affiliation:**

**Abstract Submission:**

All surfaces immersed in the marine environment are subject to the colonization by a wide range of organisms (biofouling). Formation of biofilms constitutes a crucial step of this natural process. Analysis of the metabolic production of marine biofilms could conduct to a better understanding of (i) the mechanisms of their formation (chemical interactions with surfaces), (ii) their composition (species diversity), and (iii) their development (chemical interactions with other colonizing organisms). A LC-MS-based metabolomics approach was chosen in order to face the chemical complexity of such natural matrices.

First, four marine biofilm-forming bacteria harvested from natural biofilms formed on immersed artificial surfaces (Toulon, Mediterranean Sea, France) were studied in vitro as model strains. Their metabolomes were analyzed at different stages of growth in both planktonic and biofilm cultures. Results showed, for each strain, a clear distinction of the metabolic production depending on these experimental parameters. However, the four bacteria were discriminated each other whatever these conditions, showing strain-specific metabolomes.

The Toulon bay is historically contaminated with heavy metals, such as copper, lead or zinc. In order to determine how copper pollution would affect bacterial metabolic pathways, in vitro experiments were performed with the same model strains. Analysis of the bacterial metabolic profiles led to highlight potential biomarkers whose production would be induced by the presence of copper, suggesting an adaptation of the cell metabolism in response to the exposure to this metal. Furthermore, a large study was conducted to highlight correlations between chemical, biochemical, and biological components of marine biofilms and metal contamination. Natural biofilms were first harvested from artificial surfaces immersed during a one month-field experiment at five locations along an anthropization gradient in the Toulon bay with contrasted pollution levels. Metabolomic analysis was then conducted along with characterization of microbial communities and exopolymeric substances (EPS).

**Poster #: 66**

**Abstract #: 2085**

**Abstract Title:** Maximizing the value of metabolomics data: exploring the influence of data analysis parameters on model classification and feature selection

**Authors:** Fidele Tugizimana, Ian Dubery, Paul Steenkamp, Lizelle Piater,

**Presenting Author Affiliation:** University of Johannesburg

**Abstract Submission:**

Introduction: Metabolomics studies generate information-rich, high-dimensional and complex data sets that remain challenging to handle and fully exploit. Despite the remarkable progress in the development of tools and algorithms, as presented in the recent review (Misra & van der Hooft, 2016), the 'exhaustive' extraction of information from these metabolomics data sets is still a non-trivial undertaking. Using an LC-MS-based untargeted metabolomics data, this study explored the influence of collection parameters in the data pre-processing step, scaling and data transformation on the statistical models generated, and feature selection thereafter.

Methods: ESI positive raw data generated from an LC-MS-based untargeted metabolomics study (sorghum plants infected with a fungal pathogen) were used. Raw data were pre-processed with MarkerLynx software (Waters Corporation), converting the three-dimensional LC-MS raw data into a table of time-aligned detected features (Rt, m/z and intensity) in each sample. Here two parameters were varied: intensity threshold (50-100 counts) and mass tolerance (0.05-0.1 Da). After the pre-processing, the data sets were imported into SIMCA 14 (Umetrics) for more data cleaning and statistical modelling. Here different scaling (UV, Pareto, etc.) and data transformation (log and power) methods were explored.

Results: The results showed that the pre-processing parameters (or algorithms) influence the output dataset: number of defined features. Furthermore, the study demonstrates that the pre-treatment of the data prior to statistical modelling effects the subspace approximation outcome: e.g. the amount of variation in X-data that the model can explain and predict. The results indicates also that the pre-processing and pre-treatment steps influence, subsequently, the number of statistically significant extracted/selected features.

Novel aspects: Metabolomics data are often exceedingly rich in information. This study demonstrates that understanding the structures of such data and exploration of various algorithms (and parameters), are vital and mandatory in data mining to maximize the value of the acquired data.

**Poster #: 68**

**Abstract #: 2159**

**Abstract Title:** The mechanism of action of biocides: Epicoccum derived antimicrobials

**Authors:** Alex Lee, Simon Swift, Silas Villas-Boas,

**Presenting Author Affiliation:** School of Biological Sciences, University of Auckland

**Abstract Submission:**

Epicoccaeine is a pigmented secondary metabolite produced by the fungus *Epicoccum purpurascens* as part of a yellow pigment. Unlike the other polyene antifungal agents, it is a water soluble polyene peroxide with exclusive activity against moulds but not yeasts. This is an exceptionally rare characteristic in nature as most known antifungal agents target both fungal morphotypes. Therefore, epicoccaeine is very likely to possess a novel mode of action. One way of generating the hypothesis on the mode of action of epicoccaeine is by profiling the metabolic responses of target mould cells when they are exposed to sub-lethal dosage of this compound. This data can be compared against the library of responses of the target cells when they are exposed to other antifungal compounds with known mode of action, which would give us an idea of how epicoccaeine may function. In addition to this, information obtained can later be combined with various imaging approaches and other post-genomic methodologies, including transcriptomics and proteomics, for deeper understandings in molecular mechanism of this antifungal metabolite. In order to achieve this, pigmented metabolites of the *E. purpurascens* were firstly extracted and isolated from its culture on solid media. Purification and separation of epicoccaeine from other metabolites, especially from epipyronone - an isomer of epicoccaeine with different biological activity, was conducted through solid phase extraction (SPE) and preparative HPLC. The separated compounds were analysed by LC-MS, and the purity of epicoccaeine was confirmed. The knowledge on how epicoccaeine may function will be crucial for development of the compound formulation and application methods while maintaining or improving its potential as an antifungal agent.

**Poster #: 69**

**Abstract #: 2527**

**Abstract Title:** The plasma metabolic phenotype of patients with Acute Liver Failure characterised using <sup>1</sup>H NMR and targeted UPLC-MS

**Authors:** Rabiya Zia, Nicola Gray, Vishal Patel, Ian Wilson, Julia Wendon, Mark McPhail, Muireann Coen,

**Presenting Author Affiliation:** Imperial College London

**Abstract Submission:**

Acute Liver Failure (ALF) is characterized by a rapid onset of hepatocellular injury, encephalopathy and coagulopathy. Mortality rates for ALF remain high at approximately 50%, despite significant advancement in critical-care therapies and the use of emergency liver transplantation (ELT). Most deaths occur from multi-organ failure from an abnormal host response to systemic inflammation or intracranial hypertension from hepatic encephalopathy (HE). We explore the complex mechanistic interactions between host metabolism, cell death and inflammation (“immunometabolism”) in a cohort of patients with ALF.

Metabolic phenotyping, using <sup>1</sup>H NMR spectroscopy and targeted ultra-performance-liquid-chromatography coupled with mass spectrometry (UPLC-MS) techniques, were employed to profile the plasma metabolome in ALF patients. We metabolyped 371 patients in total including those with ALF, disease controls (Acute Liver Injury, cirrhosis and severe sepsis) and healthy controls. Multivariate analysis of <sup>1</sup>H NMR data revealed a number of metabolites specifically perturbed in ALF which included lipids, amino acids (AA), and energy metabolites – citrate and pyruvate.

The targeted UPLC-MS method employed to quantify plasma AAs and profile other amino-containing compounds, revealed elevation of tyrosine, phenylalanine, and glutamine in ALF, which promote the development of HE. Furthermore, elevation of the sulphur containing AAs cystathionine and methionine indicate perturbation of the transsulfuration pathways. Additionally, inflammatory cytokine array and clinical metadata have been integrated with the metabolome using multivariate PLS regression models. Preliminary results suggest a strong correlation between TNF-α levels and N-acetylglycoprotein, providing further insights into the immuno-metabolic mechanisms present in ALF. The findings from this work could help elucidate the metabolic consequences of systemic inflammation in ALF and lead to novel biomarkers to aid selection for ELT.

**Poster #: 70**

**Abstract #: 2425**

**Abstract Title:** In-situ perfusion absorption study of rosemary compounds.

**Authors:** Álvaro Fernández Ochoa, Isabel Borrás Linares, Amudena Pérez Sánchez, Enrique Barraji3n Catal3n, David Arr3ez Rom3n, Vicente Micol, Antonio Segura Carretero,

**Presenting Author Affiliation:** Universidad de Granada

**Abstract Submission:**

The aim of this work was to study in depth the small intestine absorption and metabolism of the bioactive compounds present in a *Rosmarinus officinalis* leaf-extract with proven antiproliferative/cytotoxic activity against colorectal cancer cells. For this purpose, an in situ perfusion assay was performed in mice after the acute ingestion of the rosemary extract. Samples of gastrointestinal liquid taken at different times during the assay were properly treated and analysed by HPLC-ESI-QTOF-MS.

The results showed that most phenolic compounds, previously characterized in the rosemary extract, were found in the gastrointestinal liquid samples obtained at all times of the assay. The absorption rate coefficients ( $K_a$ , h<sup>-1</sup>) for each phenolic compound were estimated. In general, flavonoids and diterpenes showed higher absorption constants, indicating a greater absorption of these compounds in small intestine, especially compared to triterpenes. Specifically, the compounds diosmetin, genkwanin, cirsimaritin, rosmanol, carnosic acid, rosmadial, carnosol and epiisorosmanol were the compounds with the highest absorption.

Furthermore, several metabolites of the major bioactive compounds of the rosemary leaf-extract, such as 5,6,7,10-tetrahydro-7-rosmariquinone, carnosic cysteine, carnosic acid sulfate and glucuronides forms of rosmanol, carnosol and carnosic acid were found in these gastrointestinal samples. These compounds seemed to suffer a rapid metabolism owing to their early detection, appearing in the samples collected after 5 min of the starting point of the assay.

The results obtained could help to clarify the absorption and metabolism of rosemary bioactive compounds, which would contribute to a deeper understanding of the mechanism of action of these compounds against colorectal cancer. However, further studies must be carried out in order to provide a more complete view of this mechanism in humans.

**Poster #: 73**

**Abstract #: 2507**

**Abstract Title:** Targeted metabolomics of *Gammarus pulex* following controlled exposures to selected pharmaceuticals in water

**Authors:** Cristian Gómez-Canela,

**Presenting Author Affiliation:** IDAEA-CSIC

**Abstract Submission:**

The effects of pharmaceuticals and personal care products (PPCPs) on aquatic organisms represent a significant current concern. Herein, a targeted metabolomics approach using liquid chromatography-high resolution mass spectrometry (LC-HRMS) is presented to characterise concentration changes in 29 selected metabolites following exposures of aquatic invertebrates, *Gammarus pulex*, to pharmaceuticals. Method performance revealed excellent linearity ( $R^2 > 0.99$ ), precision (0.1-19 %) and lower limits of detection (0.002-0.20 ng) for all metabolites studied. Three pharmaceuticals were selected representing the low, middle and high range of measured acute toxicities (of a total of 26 compounds). Gammarids were exposed to both the no-observed-adverse-effect-level (NOAEL) and the lowest-observed-adverse-effect-level (LOAEL) of triclosan (0.1 and 0.3 mg L<sup>-1</sup>), nimesulide (0.5 and 1.4 mg L<sup>-1</sup>) and propranolol (100 and 153 mg L<sup>-1</sup>) over 24 hrs. Quantitative metabolite profiling was then performed. Significant changes in metabolite concentrations relative to controls are shown and they display distinct clustered trends for each pharmaceutical. Approximately 37 % (triclosan), 33 % (nimesulide) and 46 % (propranolol) of metabolites showed statistically significant time-related effects. Observed changes are also discussed with respect to internal concentrations of the three pharmaceuticals measured using a method based on pulverised liquid extraction, solid phase extraction and LC-MS/MS. Potential metabolic pathways that may be affected by such exposures are also discussed. This represents a preliminary study focussing on quantitative, targeted metabolomics of this lower trophic level benthic invertebrates that may elucidate potential biomarkers for environmental risk assessment.



**Poster #: 74**

**Abstract #: 2572**

**Abstract Title:** Primary and secondary metabolome associated with bast fibre development in hemp

**Authors:** Christelle Andre, Lena Fragner, Sonja Tischler, Marc Behr, Sophie Charton, Jenny Renaut, Wolfram Weckwerth, Jean-Francois Hausman,

**Presenting Author Affiliation:** Department of Environmental Research and Innovatio

#### **Abstract Submission:**

##### Introduction

Hemp (*Cannabis sativa* L.) is an annual herbaceous crop grown for both its medicinal properties and the physical quality of its fibers. Textile hemp contains less than 0.3% of tetrahydrocannabinol and can provide high amount of lignocellulosic biomass in a short time. Interestingly, the stem of this fibre crop supplies both woody and cellulosic fibers: the core is lignified, while the cortex harbors long cellulose-rich fibers, known as bast fibers. This heterogeneous cell wall composition makes hemp stem an interesting model to study secondary cell wall biosynthesis. In this work, we discuss the mechanisms leading to primary and secondary growth of hemp hypocotyls using a metabolomics approach.

##### Methods

To investigate the transition from primary to secondary growth hemp hypocotyls were analyzed at four developmental stages (in 6-, 9-, 15- and 20-day plantlets). Morphological changes in cell wall development were investigated by light microscopy combined with metabolic signatures derived from an integrated GC- and LC-MS approach.

##### Results and discussion

In the hemp hypocotyl experiment hundreds of metabolites were detected and relatively quantified at all time points. A principal component analysis was performed and revealed a clear separation of the 4 time points along PC1 (explaining 62 % of the variability). During hemp growth, a significant decrease of glutamine, monosaccharides, and polyamines (including putrescine and spermidine), was associated with an increase of sucrose and of most organic acids (malate, fumarate, succinate) of the Citric Acid Cycle, corroborating the appearance of secondary bast fibres at the last time point. With this study insights into molecular mechanisms of primary and secondary growth of hemp, in particular into bast fiber development, were obtained.

**Poster #: 75**

**Abstract #: 2371**

**Abstract Title:** Alternative animal and cell models for testing (developmental) neurotoxicity of chemicals using a metabolomic approach

**Authors:** Pim Leonards, Sara Tufi, Marja Lamoree, Juliette Legler, Jessica Legradi,

**Presenting Author Affiliation:** VU University

**Abstract Submission:**

Zebrafish are widely used as model organism in (eco)toxicology, developmental and genetic studies. Recently, this model organism is used as an alternative model for mammalian organisms to study (developmental) neurotoxicity. Other advantages of this model are for instance the small size, high-throughput, short reproductive cycle, and ex-utero development of the embryo, which all makes them very suitable for toxicological and neurogenesis studies. Another alternative model to study neurotoxicity is the freshwater pond snail (*Lymnaea stagnalis*), and especially the central nervous system (CNS) of this organism is of interest. Also a neuroblastoma cell line (SH-SY5Y cells) for neurotoxicity was investigated. This paper presents the development of a metabolomic and (developmental) neurotoxicity approach using the pond snail and zebrafish larvae. Both zebrafish and the pond snail were exposed to various pesticides and the neurotransmitter profiles were studied. The focus was on dopaminergic-andrenergic, glutaminergic-GABAnergic, serotonergic, histaminergic, and cholinergic neurotransmitters systems including their precursors and metabolites. A high sensitive analytical method (LC-MS/MS) was developed to determine neurotransmitter profiles at low levels in small sample amounts of zebrafish embryos and the CNS of the pond snail. The neurotransmitter profiles in zebrafish were studied during the period from zygote to free-swimming larvae and a two stage-dependent pattern were observed. During the first 2-3 days, before the larvae hatches to a free-swimming larvae, the precursors showed a peak, whereas the neurotransmitters increased during the whole development period. Pesticide exposures, at concentrations tested below phenotypical malformations, showed significant differences in neurotransmitter and precursor levels. Interestingly, the exposure of zebrafish and pond snail to imidacloprid showed decreased levels of acetylcholine, an effect that also occurs with acetylcholine esterase (AChE) inhibiting pesticides. Both organisms are interesting alternatives to study neurotoxicity and further research focus on mechanistic studies linking neurotransmitter changes to developmental and behavior outcomes.

**Poster #: 76**

**Abstract #: 2599**

**Abstract Title:** Strain improvement of 1-butanol producing *Escherichia coli*: A metabolomics approach

**Authors:** Sastia Putri, Toshiyuki Yamashita, Walter Lavina, Sammy Pontrelli, James Liao, Eiichiro Fukusaki,

**Presenting Author Affiliation:** Osaka University

**Abstract Submission:**

Conventional metabolic engineering techniques have made a tremendous progress in constructing non-native organisms for the production of various biofuels. However, a systematic, high-throughput, and highly efficient strategy for strain improvement is required to obtain high producing strains for industrial applications. Here, we demonstrate the utility of metabolome analysis for strain improvement of 1-butanol producing *Escherichia coli* and *Synechococcus elongatus*. A widely targeted ion LC/MS-based metabolome analysis revealed a common rate limiting step in the clostridial CoA-based production pathway. Fine tuning the expression level of the limiting enzyme in the production pathway successfully improved the productivity in *Escherichia coli* by two-fold, resulting in the highest 1-butanol productivity ever reported.

**Poster #: 77**

**Abstract #: 2434**

**Abstract Title:** The integration of physiological, proteomic, and metabolomic levels reveals new adaptive and stress-responsive mechanisms in Pinus.

**Authors:** Luis Valledor, Jesús Pascual, Mónica Escandón, Wolfram Weckwerth, María Jesús Cañal, Mónica Meijón,

**Presenting Author Affiliation:** University of Oviedo

**Abstract Submission:**

Globally expected changes in environmental conditions, mainly increased temperatures, UV irradiation, and droughts, threatens plant productivity. Despite some advances towards more tolerant varieties have been achieved in edible crops, the knowledge about the specific mechanisms mediating stress adaptative responses in Conifers are scarce. Know these pathways is crucial for designing new strategies focused on maintaining forest productivity

We studied the effect temperature and UV irradiation dosages aiming to mimic future scenarios based on current models in a time course experiment in greenhouse grown plantlets. Furthermore, the availability of a common garden, including ten origins covering North-South clinal variation and Mediterranean and Atlantic basins, allowed us to have a field system to exploit natural variation towards deciphering how Pine can adapt to different environments. Current technology for high-throughput phenotyping at the different omic levels and its integrative analysis will revolutionize the way we we understand tree biology and forest management.

Two complementary techniques (GC-MS and LC-Orbitrap-MS) were used to identify and quantify the maximum number of ions corresponding to primary and secondary metabolites. Very accurate mass, comparison to custom libraries, and in some cases MS/MS, were the strategies employed for identification. Proteins were identified and quantified following a bottom up approach, employing custom databases.

The metabolome analysis of heat and UV stresses datasets allowed both the definition of novel responsive pathways and the validation in Pine of major stress responses previously described in other plants. The integration of analysed datasets (metabolome, proteome, physiology, gene expression) provided a comprehensive picture of stress responses, proposing new metabolites and proteins closely related to specific stress adaptative responses, including kinases and proteases related to signalling and metabolic coordination. The analysis of phenotypic diversity, following a “population metabolomics” approach exploiting the common garden showed how evolution to local environment was linked to metabolome specialization to adapt to different climates.

**Poster #: 78****Abstract #: 2078**

**Abstract Title:** Chemocoding as an identification tool where morphological- and DNA-based taxonomic methods fall short: *Inga* as a case study

**Authors:** Thomas Kursar, Natasha Wiggins, Dale Forrister, Maria-Jose Endara, Phyllis Coley, James Nicholls, R. Pennington, Kyle Dexter, Graham Stone, Catherine Kidner,

**Presenting Author Affiliation:** University of Utah

**Abstract Submission:**

Cataloging the world's plant diversity has been an ongoing challenge for centuries, and because of accelerated anthropogenic extinctions, the rapid documentation of biodiversity is more critical than ever. Just over a decade ago, DNA barcodes were proposed as an alternative to morphological approaches for species identification. Although DNA barcodes have proved to be successful for most organisms, these have failed to discriminate within many species-rich genera of plants. Here, we examine how chemical fingerprinting, or chemocoding, may be particularly helpful in distinguishing confusing or closely related species in the species-rich and recently radiated Neotropical genus of trees *Inga* Mill. (Leguminosae, Mimosoideae).

Using untargeted metabolomics in combination with multivariate analysis to characterize small defense-related chemical markers, we constructed phytochemical, species-level fingerprints, which we define as chemocoding. Specifically, we compared the effectiveness of conventional DNA barcoding, next generation sequencing, and chemocoding to discriminate among closely related species of *Inga* within a single site and between sites. Our results show that chemocoding is faster and more efficient and effective than DNA-based species discrimination methods.

Given that the species is the fundamental unit of analysis for conservation, biodiversity assessment, and for understanding ecological and evolutionary processes, the development of accurate identification methods is essential. Moreover, even after species have been designated, distinct species may not differ morphologically such that correct identification in the field may require a cheap, fast method that will permit confirmation for every plant. We suggest that chemocoding is a valuable tool for distinguishing confusing species, particularly in the diverse tropics where many species-rich genera do not resolve with DNA barcoding.

**Poster #: 79**

**Abstract #: 2438**

**Abstract Title:** Contrasting plastic and evolutionary responses of *Eriophorum vaginatum* to climate warming cascade from molecular to ecosystem levels

**Authors:** Tom Walker, Lena Fragner, Luca Bragazza, Constant Signarbieux, Susan Ward, Nicholas Ostle, Brian Forde, Richard Bardgett, Wolfram Weckwerth,

**Presenting Author Affiliation:** Faculty of Life Sciences, Michael Smith Building,

**Abstract Submission:**

Anthropogenic greenhouse gas emissions have caused a rise in global temperature of 0.76 °C since the turn of the 20th Century. Plants responding to climate warming have the potential to accelerate future climate change through their control over carbon flow between ecosystems and the atmosphere. Rising temperatures elicit selective pressure on many plant species, driving genetic adaptation and shifts in plant phenotypes over evolutionary time. However, warming also stimulates phenotypic plasticity in individual plants without necessarily causing genetic adaptation. Despite this, the persistence of plasticity over evolutionary time is uncertain, and little is known about the influence of intraspecific variation on ecosystem carbon cycling. Here, we used a reciprocal transplant experiment on an elevation-based climate gradient to compare the effects of genetic adaptation to warming (i.e. provenance effects) and plasticity caused by warming (i.e. transplantation effects) on phenotypes of the plant *Eriophorum vaginatum*. We integrated approaches from metabolomics, ecophysiology and biogeochemistry to measure phenotypes at molecular, leaf and plant levels, and to elicit their impact on CO<sub>2</sub> fluxes at the ecosystem level. We show that plastic responses to warming (i.e. transplantation to low elevation) destabilise primary metabolism, potentially increasing investment in secondary metabolism. We suggest that this leads to observed higher leaf carbon content, lower nitrogen content and reduced photosynthetic performance, impairs growth, and decreases net ecosystem CO<sub>2</sub> uptake. Contrastingly, plants genetically adapted to warmer temperature (i.e. low elevation provenance) have a similar primary metabolism to plants genetically adapted to cooler temperature (i.e. high elevation provenance), display no differences in leaf carbon and nitrogen contents, show greater plant growth and do not differently influence net ecosystem CO<sub>2</sub> flux. Our findings reveal that plastic *E. vaginatum* responses to warming can influence ecosystem carbon cycling, and moreover that these responses are not echoed in the phenotypes generated by genetic adaptation to warming.

**Poster #: 80**

**Abstract #: 2211**

**Abstract Title:** Mass Spectrometry-Based Metabolite Profiling of 62 Indigenous Plant Species and Their Correlation with Bioactivities

**Authors:** Donggu Oh, Sarah Lee, Sunmin Lee, Ga Ryun Kim, Jong Seok Lee, Hee-sun Yang, Joohong Yeo, Choong Hwan Lee,

**Presenting Author Affiliation:** Konkuk University

**Abstract Submission:**

Mass spectrometry (MS)-based metabolite profiling of 62 indigenous Korean plant species was performed by ultrahigh performance liquid chromatography (UHPLC)-linear trap quadrupole-ion trap (LTQ-IT) MS/MS combined with multivariate statistical analysis. In partial least squares discriminant analysis (PLS-DA), the 62 species clustered depending on their phylogenetic family (Aceraceae, Betulaceae, Fagaceae, Asteraceae, Fabaceae, and Rosaceae). In particular, Aceraceae, Betulaceae, and Fagaceae were distinguished from Rosaceae, Fabaceae, and Asteraceae in PLS-DA score plot. Quinic acid, gallic acid, quercetin, quercetin derivatives, kaempferol, and kaempferol derivatives were identified as family-specific metabolites, and were found in relatively high concentrations in Aceraceae, Betulaceae, and Fagaceae. Fagaceae and Asteraceae were selected based on results of PLS-DA and bioactivities to determine the correlation between metabolic differences among plant families and bioactivities. Quinic acid, quercetin, kaempferol, quercetin derivatives, and kaempferol derivatives were found in higher concentrations in Fagaceae than in Asteraceae, and were positively correlated with antioxidant and tyrosinase inhibition activities. These results suggest that metabolite profiling was a useful tool for finding the different metabolic states of each plant family and understanding the correlation between metabolites and bioactivities in accordance with plant family.

**Poster #: 81**

**Abstract #: 2427**

**Abstract Title:** Data Integration & Systems Biology for Metabolomics

**Authors:** JIANGUO XIA,

**Presenting Author Affiliation:** McGill University

**Abstract Submission:**

The fast growth and increasing application of metabolomics have created several urgent bioinformatics challenges including meta-analysis of multiple metabolomics data sets for robust biomarker identification, systems biology for high-resolution untargeted LC/MS metabolomics data, as well as integration of metabolomics data with data from upstream omics platforms, etc. The MetaboAnalyst (v1.0 - v3.0) platform has been primarily developed to support comprehensive analysis of a single metabolomics data with more focus on targeted metabolomics. Here I will introduce the upcoming version of MetaboAnalyst (v4.0) that has been significantly enhanced with six new modules on data integration and systems biology for metabolomics. Some of its prominent features include: powerful network analysis and visualization to enable systems-level data interpretation for both targeted and untargeted metabolomics comprehensive support for predicting perturbed pathways and biological processes directly from untargeted LC/MS data implementation of several robust meta-analysis methods for integrating multiple metabolomics data sets support for metabolome-wide association analysis (MWAS) and finally, support for integrative analysis of metabolomics with transcriptomics (microarray or RNAseq) or metagenomics (shotgun or 16S rRNA) data. These features have been implemented using the latest web/cloud technologies to enable high-performance interactive data analysis through easy-to-use interface. We believe that this new version of MetaboAnalyst will contribute significantly to the metabolomics community.



**Poster #: 82**

**Abstract #: 2251**

**Abstract Title:** Comparative parts(leaf, twig, and fruit) of *Alnus firma* based on metabolite profiling and bioactivity

**Authors:** Sunmin Lee, Ga Ryun Kim, Donggu Oh, Jong Seok Lee, Hee-sun Yang, Joohong Yeo, Sarah Lee, Choong Hwan Lee,

**Presenting Author Affiliation:** Konkuk university

**Abstract Submission:**

The members of genus *Alnus* are well known for their traditional uses in the treatment of various symptoms such as stomachache, diarrhea, and fever. Depend on their parts, a little different treatments are performed. In this study, parts(leaves, twigs, and fruits) of *Alnus firma* were used to perform metabolite profiling by using ultrahigh performance liquid chromatography-quadrupole-orbitrap mass spectrometry (UHPLC-Q-Orbitrap-MS) combined with multi statistical analysis, and to measure antioxidant activity such as DPPH radical scavenging assay. In principle component analysis (PCA) score plot revealed the distinct three grouping patterns for each part of *Alnus firma*. Phenolic acids (quinic acid and chlorogenic acid), organic acid (citric acid), flavonoids (quercetin, naringenin, and kaempferol), and flavonoid derivatives (quercetin-3-D-galactoside, quercetin-3-D-glucoside, kaempferol-3-O- $\beta$ -rutoside, and kaempferol-3-glucoside) were identified as a significantly different metabolites separating each part of *Alnus firma*. As the result of DPPH radical scavenging assay, it showed that fruits had significantly higher antioxidant activity than leaves and twigs. This means that fruits had considerable and diverse antioxidants in comparison with leaves and twigs of *Alnus firma*. Throughout this study, we could find part-specific metabolites in *Alnus firma* and also help understanding the correlation between specific metabolites and antioxidant activity using metabolomic tool.

**Poster #: 83**

**Abstract #: 2295**

**Abstract Title:** Metabolic responses of *Eucalyptus* spp to changes in different regimes of temperature

**Authors:** Joao Mokochinski, Paulo Mazzafera, Alexandra Sawaya, Ric de Vos, Robert Hall,

**Presenting Author Affiliation:** Wageningen University and Research

**Abstract Submission:**

*Eucalyptus* spp. are considered the world's most widely planted hardwood trees and are commercially important plants. As a global renewable resource of both fibers and energy, they have been extensively used as raw material for pulp and paper industry, production of cellulose, biomaterials and bioenergy. In this context, *E. grandis* is one of the most widely cultivated species, mainly because it has adapted to a broad range of climate conditions. However, temperature is one of the factors that still limits the geographical distribution and seasonal growth of this species. For this reason, we choose untargeted metabolomics to address the metabolic response of *Eucalyptus* to different temperatures regimes.

*Eucalyptus* were cultivated at different temperatures (10, 20 and 30°C) simulating standard and extreme growth conditions. Leaf samples were extracted according to well-established protocols and analyzed using two platforms (GC-TOF MS and LC-MS LTQ Orbitrap). Statistical tools were used to evaluate differences between replicates and treatments. Identification of relevant metabolites was based on comparisons of retention time, accurate mass and MS/MS information with an in-house library.

Our findings indicate that the metabolism of *Eucalyptus* is affected by changes in temperature. Measurements on stem materials (dry mass) indicate that the temperature of 20°C increased the biomass production, suggesting this species grows better at moderate temperature. A clear effect of temperature was also observed following PCA of the metabolite profiles. Correlation between the dry mass of stems and soluble sugar content of leaves was observed, indicating that this class of compounds varies proportionally to biomass production. Secondary metabolites, identified by LC-MS, also show similar correlations with biomass production. Most of the compounds identified by LC-MS are flavonoid glycosides. For example, at 10 and 20°C we observed higher contents of grandinol, agglomerone and syringin while 30°C favors the formation of glucopyranoside derivatives.

**Poster #: 84**

**Abstract #: 2107**

**Abstract Title:** Metabolic robustness underpins a predictive mechanism of maize hybrid performance in the field

**Authors:** Francisco de Abreu e Lima, Matthias Westhues, Lothar Willmitzer, Albrecht Melchinger, Zoran Nikoloski,

**Presenting Author Affiliation:** Max-Planck Institute of Molecular Plant Physiology

**Abstract Submission:**

Heterosis, or hybrid vigor, is extensively exploited for yield gain in maize (*Zea mays* L.). Yet, the molecular mechanisms underlying heterosis remain elusive. A predictive mechanism for hybrid performance in the field based on data from early developmental stages of maize grown under controlled conditions will significantly improve the efficiency of hybrid breeding. Here we conducted a comparative metabolomics-based analysis of young roots from in vitro germinating seedlings and from leaves of field-grown plants in a large panel of inbred lines from the Dent and Flint heterotic patterns as well as selected F1 hybrids. We show that the deviation of the metabolic profiles in young roots from the representative profile of the best performing lines is predictive of biomass in the field, and that this holds, but is not as pronounced, for metabolic profiles of leaves from field grown plants. In addition, a correlation-based analysis highlights the relevance of defense-related metabolites to hybrid performance. Therefore, our findings indicate the potential of metabolic profiles from young maize roots grown under highly controlled conditions for predicting hybrid performance in the field, thus bridging the greenhouse-field gap.

**Poster #: 85**

**Abstract #: 2047**

**Abstract Title:** 1H-NMR-based metabolomics approach to understanding the influence of seasons on the metabolomic profile of *Greyia radlkoferi*

**Authors:** Noluyolo Nogemane,

**Presenting Author Affiliation:** UNISA

**Abstract Submission:**

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PhD student

1H-NMR-based metabolomics approach to understanding the influence of seasons on the metabolomic profile of *Greyia radlkoferi*

N. Nogemane, G. Prinsloo

Department of Agriculture and Animal Health, University of South Africa, Private Bag X6, Florida, 1710, South Africa

*Greyia radlkoferi*, commonly known as the mountain bottlebrush is an indigenous South African shrub or small tree growing in the northern KwaZulu-Natal (formally known as Zululand) bordering the country of Swaziland. The shrub is mostly used as a decorative tree for the inland gardens and is popular as an ornamental plant. However, a recent phytochemical study reported the potential of *G. radlkoferi* leaf extracts as formulations in the development of cosmeceutical products for the treatment of skin hyper-pigmentation. 1H-NMR coupled with multivariate data analysis was used to distinguish variations among *G. radlkoferi* leaf materials harvested from different sites during different seasons viz. spring, summer, autumn and winter. Separation into clusters according to seasons was not evident between species harvested from different sites. However, when each site was considered, a clear separation into four groups representing the four seasons was observed. Contribution plots showed the metabolites responsible for the variation in leaf material harvested in different seasons. The biological activity was evaluated to determine the influence the effect of the site and season. Considering the changes in the IC50 values and the clustering of the samples, recommendation on the most suitable harvesting time and area have been developed.

**Poster #: 86**

**Abstract #: 2361**

**Abstract Title:** Towards the Circadian Drosophila Metabolome

**Authors:** Stefan Schaebler,

**Presenting Author Affiliation:** German

**Abstract Submission:**

Circadian clocks play a major role in orchestrating metabolism, physiology and behavior in almost all organisms. They have been extensively studied and are perhaps best understood on the levels of gene expression and behavior. Little is known, however, on the daily timing of metabolism. To address this question we use *Drosophila melanogaster* as a model organism due to the broad variety of mutants.

Flies were raised under 12-h/12-h light/dark (LD) and ad libitum feeding conditions. Prior to analysis, flies were separated into heads and the other body parts (referred to as body), pooled (5 heads/bodies per sample) and extracted using a liquid/liquid partitioning protocol. Metabolite analysis was performed using different UPLC-ESI-qTOF-MS methods. Two approaches were applied to identify clock gene-dependent and circadian regulated metabolic pathways. In one approach we determined the metabolic difference between wild type Canton-S (CS) and arrhythmic *per0* flies at two time points (TP). In another approach, we determined the metabolome of wild type flies collected at different times (12TPs/d 3d) throughout the LD cycle and used the JTK Cycle algorithm to identify rhythmic metabolites.

The first approach revealed a major impact of the *per* gene on metabolism. Levels of about 7% and 11% of all detected metabolites in heads and bodies, respectively, were significantly different in *per0* vs CS flies at two TPs under LD conditions. The second approach revealed more than hundred oscillating metabolites. Interestingly, rhythmicity is more pronounced in head compared to body samples.

Those results are a first indication for the importance of a functional clock for metabolism.

**Poster #: 87**

**Abstract #: 2545**

**Abstract Title:** Effect of drought stress on amino acid metabolism of model plant *Brachypodium distachyon*

**Authors:** Ebru Yilmaz, Kerem Kaya, Bahar Sogutalmaz Ozdemir, emrah nikerel,

**Presenting Author Affiliation:** Yeditepe University

**Abstract Submission:**

Metabolomics aims to systematically gather (quantitative) information on metabolites in the cell and is commonly viewed as the “missing link” between genomics, transcriptomics and physiology. As such, it constitutes one of the important pillars in understanding environmental effects on plants.

*Brachypodium distachyon*, native to the Mediterranean and Middle-east region, is a member of the Pooideae subfamily of the family Poaceae which also includes the small grains such as wheat, oat and rye. This close relationship makes *Brachypodium* remarkable as model organism for investigation of crop species. While other crops relatively have complex genomes and unfavorable growth conditions, *Brachypodium* has all the characteristics to be a tractable model organism such as having a compact genome (~272 Mbp), short generation time (12 weeks), 5 pair of chromosomes ( $2n=10$ ), the ability of self-pollinate and simple growth requirements. In recent years, despite of an increasing interest in genomic and transcriptomic studies, metabolomics studies for *B. distachyon* are at their infancy.

Environmental disasters, climate change and other stress factors including cold, drought, heavy metals or salinity, with limited land resources available for agricultural expansion, significantly decrease the crop productivity. Particularly, drought which can be described basically as water deficiency or incapable access to water is one of the greatest abiotic stress factors rapidly increasing and threatening agriculture on a global scale.

The scope of the current study is to quantify the effect of drought stress on model plant *Brachypodium distachyon*, in particular on amino acid metabolism. In doing so, the intracellular amino acid levels, extracted from stress treated leaves and subsequently derivatized, will be quantified using GC-MS platform. The amino acid levels obtained from treated and non-treated samples will be compared, for functional analysis to gain further insights into the effect of this abiotic stress on agriculturally important crops, such as wheat and barley.

**Poster #: 88**

**Abstract #: 2271**

**Abstract Title:** Effector-triggered immunity (ETI) of *Brassica oleracea* to the infection of *Xanthomonas campestris* pv. *campestris*

**Authors:** María Tortosa, Pablo Velasco, Elena Cartea, Víctor Rodríguez,

**Presenting Author Affiliation:** Misión Biológica de Galicia (CSIC)

**Abstract Submission:**

Black rot, considered one of the most destructive diseases of *Brassica* crops, is caused by the bacterial pathogen *Xanthomonas campestris* pv. *campestris* (Xcc). Despite of its economic repercussion, little is known about the infection development and *Brassica* defense mechanisms against this bacteria. During a well established bacterial infection, pathogen effectors are recognized by specific plant proteins that triggers the effector triggered immunity (ETI), which results in an accelerated disease resistance. With the aim of understanding the physiological processes triggered during late Xcc response, we analyzed the metabolome of *Brassica oleracea* plants at 6 and 12 days post-infection (dpi) by using an LC-ESI-Q-TOF. Fifty-seven and 223 features are accumulated differentially between control and inoculated plants at 6 and 12 dpi, respectively. Surprisingly, the response at 6 dpi is characterized by a significant decrease of phenolic compounds such as glycosylated or glycosylated-acylated flavonoids, compounds that use to act as precursors of molecules involved in the plant defense systems. Besides, there is an increase of glycerophospholipids which may suggest an activation of lipid signaling. In contrast, the response at 12 dpi is characterized by a strong increase of phenolic compounds including salicylic acid, a well-known key phytohormone involved in both local and systemic resistance. Likewise, high levels of Tryptophan were observed the Tryptophan biosynthetic pathway provides precursors for plant defense-related secondary metabolic compounds and is known to be induced by pathogens. This work reports the relationship between secondary metabolism and Xcc infection in *B. oleracea* plants and provides new insights into aspects of plant-pathogen interaction.

**Poster #: 89**

**Abstract #: 2511**

**Abstract Title:** New Insights on Metabolite Identification: MS fragmentation, adduct formation and quantum chemistry

**Authors:** Mpho Makola,

**Presenting Author Affiliation:** University of Johannesburg

**Abstract Submission:**

One of the challenges in metabolomics studies is the confident identification of metabolites within the biosystem under study. These large groups of structurally diverse small molecules form part of the metabolome of the biological system and the complexity of the metabolome is amplified by the stereochemistry of the metabolites. Current analytical methodologies are still limited in confidently identifying all these metabolites (i.e. full characterisation to the stereochemistry level). In this study, the caffeoylquinic acids (CQAs) were used to develop annotation methods with high confidence: drawing insights from fragmentation, adduct formation and quantum chemistry. The CQA molecules provide a classic example of this structural diversity, with the presence of regional and geometrical isomers. Although ion-trap MS techniques have been applied for the annotation of the regional isomers, the accurate differentiation of geometrical isomers is still almost impossible.

UV-irradiated methanolic solutions of the CQAs were analysed using a UHPLC-QTOF-MS method in negative ionisation mode. An in-source collision induced dissociation (ISCID) method was optimised by varying both the capillary and cone voltages to achieve differential MS fragmentation patterns between geometrical isomers of the CQAs. Density functional theory (DFT) models were used to optimise the diCQA structures and to determine the metal binding interactions between sodium and the diCQA geometrical isomers.

The optimised ISCID method induced differential fragmentation patterns between the geometrical isomers of the diCQA molecules based on their alkali metal binding characteristics. In this method, the cis isomers had a consistently greater sodium adduct peak than the trans isomer. From the computed binding energies of each isomer, the cis isomers were further differentiated from one another.

This study developed an optimised ISCID based method, combined with metal adduct formation, which can be used to differentiate between geometrical isomers. Furthermore, computational modelling helped between the cis isomers based on their metal binding energies.



**Poster #: 90**

**Abstract #: 2026**

**Abstract Title:** UHPLC-MS analysis of extracts of guaco (*Mikania glomerata* Spreng. and *Mikania laevigata* Schultz Bip. ex Baker) submitted to different drying procedures

**Authors:** Alexandra Sawaya, Alexandre Borghi,

**Presenting Author Affiliation:** UNICAMP, Institute of Biology

**Abstract Submission:**

Two species of medicinal plants: *Mikania glomerata* Spreng. and *Mikania laevigata* Schultz Bip. ex Baker, are known as guaco in Brazil and popularly used for colds and asthma. The ethanolic extract of the dried leaves is commercially incorporated in the formulation of cough syrup. However, the drying procedure can affect the active components of these extracts. Leaves of both species were dried by lyophilization oven dried at 40°C for two days and dried in the shade at ambient temperature for two weeks. Samples were collected periodically (in triplicate) during the procedure and frozen (-80°C). At the end of the collection period all samples were lyophilized, ground and extracted with 70% ethanol. Their composition was analyzed by ultra-high efficiency chromatography coupled to mass spectrometry (UHPLC-MS). Contents of coumarin, umbelliferone, o-coumaric acid, chlorogenic acid, dicaffeoylquinic acid, tricaffeoylquinic acid, kaurenoic acid, grandifloric acid and cupressenic acid were quantified and compared between samples. Significant differences were determined by the Tukey test ( $p < 0.05$ ). Although the chemical marker for these species is only coumarin, all these compounds are potentially involved in the biological activity of their extracts.

The oven dried leaves presented a more similar composition to the leaves directly dried by lyophilization, which was considered the control of the fresh leaves and had the highest content of active compounds. The leaves which were dried at ambient temperature in the shade presented a greater modification in composition, possibly due to enzymatic and microbial degradation. Therefore, for these species, oven drying at a low temperature (40 °C) is the best method to ensure adequate therapeutic activity of their extracts, as lyophilization of large amounts of leaves is not economically feasible.

**Poster #: 91**

**Abstract #: 2162**

**Abstract Title:** Integrating metabolomics and micrometeorology to assess the regulation of cluster sunlight exposure as a tool to improve wine quality in arid to semi-arid regions

**Authors:** Noam Reshef, Aaron Fait, Nurit Agam, Natasha Walbaum,

**Presenting Author Affiliation:** Ben Gurion University

**Abstract Submission:**

Ongoing expansion of agriculture to marginal areas combined with global warming and desertification are increasing the surface area of vineyards experiencing high solar radiation and daily temperatures. Under these conditions, over-exposure of the clusters to sun irradiance is common, leading to elevated berry temperature and risk of sun-burn which accelerates the degradation of quality-related secondary metabolites.

Modern viticulture is still largely based on guidelines constructed under a temperate climate and fertile soils, where open canopies and exposed clusters are required in order to minimize rot damage and induce the accumulation of pigments and aroma compounds. Our aim is to define a new set of practical guidelines to face the challenges associated with warm-climate viticulture on yield and wine quality.

In this study, we applied photo-selective shading nets with 30 and 60% shading intensities and different spectral properties (neutral, blue and red light enrichment) around the cluster-zone in order to control light intensity and spectrum arriving to the clusters with minimal effect on other vine tissues. We integrated continuous micro-climatic measurements with fruit metabolic profile to provide in-depth description of the modulation of berry micro-climate by the use of shading, correlate fruit metabolism with micro-climate parameters, study the effect of specific light spectrum shift on the accumulation of skin flavonoids and derive the optimal conditions required to obtain high quality fruits in semi-arid regions.

Photo-selective shading affected cluster-zone light spectrum in the visible and infra-red (IR) segments. Incoming irradiance intensity negatively correlated with berry titratable acidity and weight. Shading intensity affected the metabolic profile of skin anthocyanins, flavanols and flavonols and the ratio of ortho-diphenol flavonoids with a consequent effect on wine color intensity and hue.

Our data suggests that regulation of cluster sun exposure can mitigate the consequences of warm climate on fruit composition and improve final wine quality.

**Poster #: 92**

**Abstract #: 2553**

**Abstract Title:** Dose-dependent and time-dependent metabolic dynamics of *Chlamydomonas reinhardtii* under phosphate-deprivation

**Authors:** Cheol-ho Jang,

**Presenting Author Affiliation:** kookmin University

**Abstract Submission:**

Algal blooming provokes an environmental disaster in the world including Korea. Thus scientists have been investigating the methodologies to inhibit or limit algal growth by depriving key nutrients. Particularly phosphates are the crucial limiting factor in fresh water plant and algal growth. Thus we explored phosphate-linked metabolic dynamics in dose- and time-dependent manner using attractive model organism, *Chlamydomonas reinhardtii*. We timely resolved the temporal modulation of the algal metabolomics according to different growth phases and simultaneously monitored the metabolic responses to different concentration of phosphate in culture media (gradual increase in nutritional stress). Further, we systematically characterized the metabolic snapshot at the levels of metabolic pathway and network, which may mirror the dysfunctionality of “flux”.

**Poster #: 93**

**Abstract #: 2202**

**Abstract Title:** Combining LCMS data from two years for plant performance biomarkers discovery

**Authors:** Maria Urrutia, Stephane Bernillon, Nadia Lamari, Mickaël Maucourt, Patricia Ballias, Hélène Sellier, Yves Gibon, Catherine Giauffret, Annick Moing,

**Presenting Author Affiliation:** INRA - UMR1332 Fruit Biology and Pathology

**Abstract Submission:**

Metabolomics is a useful tool to find biomarkers of plant performance in challenging conditions. Open field experiments from different years are a way to characterize robust biomarkers since experimental conditions will be slightly different. However, running metabolomics analyses in yearly batches leads to uncontrolled bias which is detrimental for the following statistical analyses. The aim of the study was to evaluate data pre-processing strategy to avoid these drawbacks and allow detecting reliable biomarker candidates.

Experiments were designed to study cold tolerance in maize crop. For two consecutive years (2013 and 2014), maize seeds were sown at two dates (April and May). Leaves of young plants were harvested and their extracts were analysed by LC-QTOF-MS. Data matrices for each year separately, or combined years were obtained using XCMS (Smith et al. 2006).

QC samples were influenced by a drift in MS response. Correction was then performed using linear models when needed. In addition, another correction was applied using 2013 QC run within 2014 analysis set to compare years. Multivariate analyses were used to detect biomarkers in each year, or combined years data.

Acknowledgements: AMAIZING (ANR-10-BTBR-01) and MetaboHUB (ANR-11-INBS-010) projects

**Poster #: 94**

**Abstract #: 2269**

**Abstract Title:** Early metabolomic response of Brassica oleracea plants against Xanthomonas campestris pv. campestris

**Authors:** María Tortosa, Marta Francisco, Pilar Soengas, Pablo Velasco,

**Presenting Author Affiliation:** Misión Biológica de Galicia (CSIC)

**Abstract Submission:**

Brassica crops form an important family of vegetables which are widely grown worldwide. These crops are affected by several diseases, of which black rot caused by Xanthomonas campestris pv. campestris (Xcc) has been reported to be one of the most devastating. Understanding the biochemical mechanisms occurring during pathogenesis is essential for the development of tools capable of coping with the disease. On this basis, Brassica oleracea plants were inoculated with Xcc race 1 and the inoculated leaves were analyzed at three times, 24, 48 and 72 hours post inoculation (hpi) by using a LC-ESI-Q-TOF. Assuming a p-value 1 as a threshold, differentially expressed metabolites were detected between infected and control plants. During the three times, 354 metabolites were differentially expressed between conditions, 251 of them over-expressed and 103 down-regulated. Most of these metabolites were detected at 48 and 72 hpi, suggesting that an interval of 24 hours is not enough to trigger biochemical defenses. Among all the metabolites identified, an increase of auxins (IAAs), brassilexin and N-Acyl homoserine lactones (N-AHLs) may be highlighted due to their biological implication. Despite of auxins have been traditionally associated with plant growth, recent studies suggest that this phytohormone also affects disease outcomes indirectly through effects on development. Regarding to brassilexin, it is a class of phytoalexin that was first described in Brassica crops, which possess a well-known role against pathogens attack. Finally, N-AHLs are a class of signaling molecules produced by gram-negative bacteria, as Xcc, responsible of quorum sensing mechanisms. By this mechanism, bacteria are able to express specific genes in response to population density. This study reports a general view of metabolomic pathways triggered during an early Xcc infection, which represents an important step toward understanding Brassica-Xcc interaction.

**Poster #: 95**

**Abstract #: 2464**

**Abstract Title:** Amino Acid-Profiling of Breast Cancer Test Subject Samples by GC-MS

**Authors:** Juan Carreño, Mónica Cala, Julián Sánchez, José Guio, Wolfram Baumann, Roland Meesters,

**Presenting Author Affiliation:** Universidad de los Andes

**Abstract Submission:**

Metabolomics is a branch of systems biology focused on the identification, quantification and interpretation of the metabolites and their impact on metabolic pathways. It has different approaches differentiated by the degree of depth of the analysis performed. This procedure has proven to be increasingly useful when working hand in hand with medicine. Different methods for diagnosis of diseases have been produced via metabolomics research.

When taking a look at cancer research, it is noticeable that a diagnosis of the disease at an early stage is crucial for a chance of a better treatment as well as a better prognosis. Metabolism of cancer subjects shows altered amino acid relative concentrations when compared to healthy subjects. The aim of this work therefore is to look for such significantly altered concentrations using Gas Chromatography Mass Spectrometry techniques.

Plasma and Urine samples from 35 breast cancer test subjects (collected by the Liga contra el Cancer, Seccional Bogotá) and 35 from control test subjects have been collected and were prepared and analyzed using an EZ-FAAST Amino Acid kit purchased from Phenomenex, following their procedure. It includes an extraction of the amino acids followed by their volatilization, and a suitable column for analysis. The kit allows for an easy and fast analysis of a total of 32 amino acids, amino acid derivatives and some other metabolites provided as standard solutions with the kit.

The results of the study of plasma samples from breast cancer test subjects will be compared to those from healthy control test subject plasma samples and will be discussed comparing them to literature results.

**Poster #: 96**

**Abstract #: 2225**

**Abstract Title:** Alterations in Plasma Lipid Profiles from Breast cancer Patients

**Authors:** Julian Aldana, Mónica Cala, Julián Sánchez, Ismael Guio, Roland Meesters, Wolfram Baumann,

**Presenting Author Affiliation:** Los Andes University

**Abstract Submission:**

Lipids are biochemical intermediates that play an important role in cellular homeostasis, including cell cycle regulation, redox balance, cell signaling and energy storage. Hence, it is not surprisingly observing alterations of the lipid profiles in serum [1], plasma [2] and urine [3] from breast cancer patients. In the present study a lipidomic approach was performed using plasma samples from a cohort consisting of 35 breast cancer subjects and 35 healthy controls. Lipids were prior to mass spectrometric analysis, extracted by MTBE using the protocol published by Matyash et al [4]. After extraction a reversed-phase liquid chromatography separation and analysis by high resolution accurate mass spectrometry (RPLC-HRAMS) was performed. Principal component analysis (PCA) and partial least-square discriminant analysis (PLS-DA) indicated statistically relevant differences between both studygroups, corresponding to glycerophospholipids including phosphatidylethanolamines, phosphatidylcholines, and lysophosphatidylcholines species. In conclusion, our results indicated that glycerophospholipids could be involved in the characteristic phenotype of breast cancer and they could be potential biomarker in discriminating breast cancer plasma samples from healthy controls plasma samples.

[1] Hammad, L.A., et al., Elevated levels of hydroxylated phosphocholine lipids in the blood serum of breast cancer patients. *Rapid Communications in Mass Spectrometry*, 2009. 23(6): p. 863-876.

[2] Qiu, Y., et al., Mass spectrometry-based quantitative metabolomics revealed a distinct lipid profile in breast cancer patients. *Int J Mol Sci*, 2013. 14(4): p. 8047-61.

[3] Kim, H., et al., Quantitative analysis of phosphatidylcholines and phosphatidylethanolamines in urine of patients with breast cancer by nanoflow liquid chromatography/tandem mass spectrometry. *Analytical and Bioanalytical Chemistry*, 2009. 393(6-7): p. 1649-1656.

[4] Matyash, V., et al., Lipid extraction by methyl-terf-butyl ether for high-throughput lipidomics. *Journal of Lipid Research*, 2008. 49(5): p. 1137-1146.

**Poster #: 97**

**Abstract #: 2122**

**Abstract Title:** Metabolic Reprogramming in Mutant KRAS Colorectal Cancer Cells

**Authors:** Dorna Varshavi, Dorsa Varshavi, Nicola McCarthy, Jeremy Everett,

**Presenting Author Affiliation:** University of Greenwich

**Abstract Submission:**

Metabolic Reprogramming in Mutant KRAS Colorectal Cancer Cells

Dorna Varshavi<sup>1</sup>, Dorsa Varshavi<sup>1</sup>, Nicola McCarthy<sup>2</sup> and Jeremy Everett<sup>1</sup>

1. Medway Metabonomics Research Group, University of Greenwich, Chatham Maritime, Kent ME4 4TB.
2. Horizon Discovery, 7100 Cambridge Research Park, Waterbeach CB25 9TL

Colorectal cancer (CRC) is one of the leading causes of cancer-related death worldwide [1]. KRAS (Kirsten Rat Sarcoma Viral Oncogene Homolog) mutations occur in approximately one-third of CRC tumours and have been associated with poor prognosis and resistance to therapeutics [2]. Despite KRAS mutants being known driver mutations, KRAS has proved difficult to therapeutically target, necessitating the identification of KRAS upstream and downstream targets. Thus, a comprehensive understanding of the molecular mechanisms underlying KRAS-driven cellular transformation is needed.

Here, we applied an exploratory, NMR-based metabonomics approach to obtain an understanding of metabolic dysregulation driven by KRAS mutations. We compared WT-KRAS and mutant KRAS effects on cancer cell metabolism using metabolic profiling of the parental KRAS G13D/+ HCT116 cell line and its isogenic derivative cell lines KRAS +/- and KRAS G13D/-. A clear discrimination was observed not only between KRAS mutants (KRAS G13D/+, KRAS G13D/-) and wild-type (KRAS +/-) but also between KRAS G13D/+ and KRAS G13D/-. The findings of this study provide potential prognostic markers and targets, which might aid the development of effective therapies against oncogenic KRAS.

**References:**

1. Ferlay J, Shin H-R, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *International Journal of Cancer*, 127(12), 2893-2917 (2010).
2. Arrington AK, Heinrich EL, Lee W et al. Prognostic and Predictive Roles of KRAS Mutation in Colorectal Cancer. *International Journal of Molecular Sciences*, 13(10), 12153-12168 (2012).



**Poster #: 98**

**Abstract #: 2231**

**Abstract Title:** Discovering Diabetic Lipid Biomarker Using HRAM LC-MS-MS Approach on a High Field Hybrid Quadrupole-Orbitrap Mass Spectrometer

**Authors:** Reiko Kiyonami, Elena Sokol, David Peak, Ken Miller,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

Lipids play a key role in cell, tissue and organ physiology with diseases such as cancer and diabetes as these diseases involve disruption of their metabolic enzymes and pathways. Identification of unique lipid biomarkers to distinguish healthy humans compared to those with a disease can have an impact on the early detection of diseases and personalized medicine. Recent advances in HPLC-MS platforms have allowed for rapid and sensitive detection of variety of lipid species with minimal sample preparation. However, challenges still remain. HPLC separation cannot separate all isomeric or isobaric molecular ions in biological sample because of the diversity in structures and physical / chemical properties of the lipidome. In order to minimize interference from the co-eluting species, a high resolution, accurate mass (HRAM) mass spectrometer is required for accurate lipid molecular ion determination. Additionally, molecular weight information alone is not sufficient for identifying each isomer of individual lipid species. MS/MS information is further required for unambiguous identification of each individual lipid species in biological samples. A high field hybrid quadrupole-Orbitrap mass spectrometer can address these challenges by delivering high mass accuracy using ultra-high resolution (up to 240,000) and acquiring large number of MS/MS data (scan speed up to 18 Hz). This allows for large number of lipid identification in a single HPLC/MS/MS run, thus enabling improved lipidome coverage. Here we demonstrate that HRAM LC MS<sup>n</sup> approach on a high field hybrid quadrupole Orbitrap mass spectrometer enables rapid putative diabetic biomarker discovery through lipidomics profiling experiments. Two phenotypes of the rat (ZDF vs. lean wild type) plasma samples were used as a model case.

**Poster #: 99**

**Abstract #: 2228**

**Abstract Title:** Facilitate Biomarker Discovery Using Integrated “Omics” Differential Analysis with High Resolution Accurate LC/MS Approach

**Authors:** Reiko Kiyonami, Sergei Snovida, Devin Drew, David Peak, Julian Saba, Andreas Huhmer, Ken Miller,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

HPLC-MS platforms are increasingly used for large scale “Omics” (proteomics, lipidomics, metabolomics, glycomics) experiments to discover new biomarkers for early disease diagnosis. Each “omics” data set provides valuable insights into biomarker candidates. However, no single omics approach is sufficient to explain the complexities associated with biological system. A better strategy is to examine the different layers of information that various omics approaches provide. Integrating multiple “omics” data will help to understand the system behavior as a whole for unravelling biological regulatory mechanisms to define the emergent properties and help to verify biomarker candidates from each “omics” workflow. Here we conducted proteomics, lipidomics and glycomics studies on phenotypical vs. normal rat plasma (Zucker diabetic fatty rat and lean rat) using Orbitrap based LC/MS platforms. The lipid profiling for the rat plasmas is performed by UHPLC-MS using a C30 column (2.1x150mm, 3µm) and a quadrupole Orbitrap™ mass spectrometer. The proteomics profiling for the rat plasmas is performed by nano LC-MS using an easy spray column (75µm x 50cm, 2µm) and a tribrid Orbitrap mass spectrometer. The same set up was used for the glycomics study where the released glycans were permethylated and analyzed by the Thermo Scientific™ Orbitrap Fusion mass spectrometer. Thermo Scientific™ LipidSearch™ 4.1 and Proteome Discoverer 2.2™ software are used for lipidomics and proteomics profiling respectively. Simglycan™ software from PREMIER Biosoft is used for glycomics profiling.

The lipid species and proteins which showed significant fold changes are determined from the profiling experiments, respectively. The correlation of phospholipids and triglycerol lipids with the lipids transfer proteins and Lipoproteins are evaluated and integrated results are reported.

**Poster #: 100**

**Abstract #: 2544**

**Abstract Title:** Serum global metabolomics approach to examine non-small cell lung cancer

**Authors:** Pawel Derezinski, Agnieszka Klupczynska, Timothy Garrett, Vanessa Rubio, Wojciech Dyszkiewicz, Zenon Kokot,

**Presenting Author Affiliation:** Poznan University of Medical Sciences

**Abstract Submission:**

Lung cancer is characterized by high mortality rates resulting from poor clinical manifestation and low effectiveness of diagnostic tools. Therefore, there is a need for identification of clinically useful markers, which would improve detection of this tumor in its early stages. Abnormalities in concentrations of multiple metabolites from different groups were observed in the blood of lung cancer patients, often even at an early stage of the disease. The aim of the study was to investigate the alterations in serum metabolome in non-small cell lung cancer (NSCLC) in Polish population using UHPLC-Q-Orbitrap-HRMS metabolomics approach.

The studied group consisted of 60 patients with newly diagnosed NSCLC (30 squamous cell carcinomas, 30 adenocarcinomas). The control group comprised 25 healthy subjects. The sera were collected after an overnight fast, stored at -80 °C until use and extracted for analysis. Global metabolite profiling was performed using a high-resolution, accurate-mass Q Exactive Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific) coupled to a UHPLC UltiMate 3000 (Dionex). Spectrometer was operating in full MS mode within the range of 70-1000 m/z. Each sample was injected twice (acquisition in positive and negative ionization). Additionally, several pooled samples representing different groups were analyzed in data dependent MS/MS mode. The obtained data was processed and the resulting metabolite profiles were subjected to multivariate statistical tests.

The results proved that the metabolic snapshot approach is suitable for examining changes in serum metabolome caused by NSCLC. Interestingly, the PCA scores plot obtained for positive ionization feature list shows better separation between NSCLC patients and controls comparing to negative ionization. Further investigation in serum global metabolite patterns in patients with NSCLC and comparison to controls is being performed in order to assess the effects of cancer type and stage on the relative concentrations of metabolites as well as to identify significant differentially expressed metabolites.

**Poster #: 101**

**Abstract #: 2022**

**Abstract Title:** A Snapshot into a Cancer Cell

**Authors:** Hannah Johnston,

**Presenting Author Affiliation:**

**Abstract Submission:**

The Warburg effect describes the survival advantage of cancer cells in that they can proliferate under low oxygen conditions via a less efficient pathway known as glycolysis. This process provides the key carbon precursors needed for cell proliferation, nucleotide and amino acid biosynthesis. The complete picture for this redirection of metabolism and increased survivability of cancer cells remains elusive. The tumour microenvironment can have a profound effect on biological functions such as transcription regulation, epigenetic alterations and post-translational modifications, all of which play an important role in the shift of cellular metabolism toward glycolysis. Tumour progression, metastasis and therapeutic resistance are associated with hypoxia, therefore comparing the metabolic fingerprint of a normal cell to a cancerous cell can help provide biomarkers for anticancer therapy. Our newly developed novel-class of SERS nanoparticles can quantitatively and non-invasively measure the redox potential of cancer cells in vitro. An analytical approach using  $^1\text{H}$  NMR spectroscopy, mass spectrometry and multivariate analysis can provide a comprehensive snapshot of the metabolic profile alterations caused by cancer. These techniques combined can help us to better understand the metabolomics and thermodynamic factors underpinning redox signalling at different redox potentials.

**Poster #: 102**

**Abstract #: 2317**

**Abstract Title:** Metabolite measurements enhance our understanding of the immunooncology portfolio

**Authors:** Filippou Michopoulos, Emma Davies, Hannah Brown, Alwin Schuller, Richard Woessner, Susan Critchlow,

**Presenting Author Affiliation:** Senior scientist

**Abstract Submission:**

Metabolite profiling analysis has been routinely used across many disease areas of interest to assess metabolic perturbations and enable pre-clinical marker identification and validation, measure target engagement and improve our understanding of wider metabolic impact of therapeutic interventions. In recent years the immune system has been recognised as a key player in cancer biology area. Here we will describe our innovative method to measure metabolic intermediates associated with immuno-suppressive pathways such as arginine and tryptophan metabolism. Platform evaluation has been undertaken using a variety of biospecimens collected from CT26 syngeneic xenograft model such as plasma, urine, tumour and liver tissues. Furthermore the results of an in vivo study evaluating the impact of IDO inhibition on the CT26 syngeneic xenograft model confirmed target engagement and metabolic perturbation in both plasma and tumour extracts.

**Poster #: 103**

**Abstract #: 2062**

**Abstract Title:** Metformin Targets PHGDH And Lowers 2-HG In Inhibiting Breast Cancer Cell Proliferation

**Authors:** Sehyun Oh,

**Presenting Author Affiliation:** Seoul National University

**Abstract Submission:**

The biguanide drug metformin is widely used for the treatment of type 2 diabetes, and now there is growing evidence supporting metformin as an anticancer drug. Here we tried to find a mechanism that can explain the phenomenon. To test the anti-cancer effects of metformin, it was treated to human breast cancer cell line MCF-7. The growth inhibitory effect of metformin in MCF-7 was observed by MTT assay, but it was not so effective to non-tumorigenic human breast epithelial cell line MCF-10A. With metabolomic analysis using NMR and LC-MS, we found various metabolic alterations, and, in particular, a decrease in 2-hydroxyglutarate (2-HG), a well-known oncometabolite. However, MCF-7 proved to be wild type for IDH1 and IDH2. As recent results suggested phosphoglycerate dehydrogenase (PHGDH) can also produce 2-HG, we investigated its involvement.

Both real-time PCR and western blot analyses showed metformin-treatment reduced the level of PHGDH, which is consistent with reduction of 2-HG observed by LC-MS. These were also corroborated by observing the decrease in 2-HG level in PHGDH-knockdown cells using siRNA. Furthermore, histone methylation level was decreased in metformin treated MCF-7 cells. These results suggest that metformin can exhibit its anti-cancer effect by downregulating PHGDH followed by the reduction of 2-HG. We also observed that 2-HG levels are higher in a set of human breast cancer tissue samples. Currently, activation of AMPK pathway by inhibiting complex I electron transport chain in mitochondria is known as the major mechanism of action of metformin. Our results suggest that metformin may have therapeutic effects in breast cancers overproducing 2-HG through PHGDH.

**Poster #: 104**

**Abstract #: 2099**

**Abstract Title:** Mutants KRAS exhibit different metabolic responses to PI3K inhibition in non-small lung cancer cells: implication for therapeutic susceptibility

**Authors:** Laura Brunelli, Elisa Caiola, Mirko Marabese, Massimo Broggin, Monica Lupi, Roberta Pastorelli,

**Presenting Author Affiliation:** IRCCS-Istituto di Ricerche Farmacologiche Mario Ne

**Abstract Submission:**

Non-small-cell lung cancer NSCLC is the leading cause of cancer-related death and accounts for over a million deaths per year worldwide. The 5-year survival rate for NSCLC is currently below 20%, highlighting the need for new treatment strategies. KRAS mutations in NSCLC patients are considered a negative predictive factor and indicate poor response to anticancer treatments. Evidences showed that KRAS mutations lead to the activation of PI3K/akt/mTOR pathway, whose effective inhibition remains a challenging clinical target. Since PI3K/akt/mTOR pathway and KRAS oncogene mutations have both a role in cancer cell metabolism, we investigated whether the activity of PI3K/akt/mTOR inhibitors (BEZ235 and BKM120) in cells harboring different KRAS status is related to their effect at metabolic level. Isogenic NSCLC cell clones expressing wild-type (WT) and mutated form (G12C, the most representative mutation in NSCLC patients) of KRAS were used to determine the response to BEZ235 and BKM120. Metabolites were measured using a targeted mass spectrometry-based quantitative metabolomic approach that included acylcarnitines, aminoacids, biogenic amines, glycerophospholipids, sphingolipids, and sugars. Metabolomics data highlighted the impairment of glutamine and serine metabolism in KRAS-G12C and KRAS-WT respectively, after pharmacological blockade of the PI3K signaling, although the net effect on cell growth, cell cycle distribution and caspase activation was similar. PI3K inhibitors caused autophagy in KRAS-WT isoform, but not in KRAS-G12C, where a striking decreased in ammonia production was found as probable consequence of glutamine metabolism impairment.

The present results provide important insights into the characterization of the metabolic rewiring associated with G12C KRAS mutation to identify new metabolic targets to enhance the response to classical PI3K/akt/mTOR pathway inhibitors in NSCLC. This study would set the basis for more effective therapeutic combinations possibly discriminating between wild-type and mutated KRAS cancer cells in NSCLC to open new potential therapeutic possibilities in NSCLC current chemotherapy.

**Poster #: 105**

**Abstract #: 2293**

**Abstract Title:** Untargeted LC-TOFMS -based metabolomics for breast cancer biomarkers: A pilot study

**Authors:** José Pérez, Caridad Díaz-Navarro, Ana Laura Ortega-Granados, Sergio Granados-Principal, Maria Ruiz, Mónica Fernández, Nuri El Azem, Francisca Vicente, Olga Genilloud, Pedro Sánchez-Rovira,

**Presenting Author Affiliation:** Fundación MEDINA, Centro de Excelencia en Investig

**Abstract Submission:**

Breast cancer is the most common cancer among women and the second cause of cancer death. Metabolomics, the unbiased identification and quantification of small molecule metabolites in biological samples, is playing a substantial role in characterization of biochemical phenotypes in pathological situations and it can be used to help in the development of new biomarkers. In this project, we have investigated the metabolic profile of patients and healthy controls in order to identify potential biomarkers of breast cancer using untargeted metabolomics methods. Plasma samples collected from 74 breast cancer patients and 20 healthy women were analyzed by LC-TOFMS using reverse phase chromatography in the positive and negative ionization mode. The receiver operator characteristic (ROC) curves analysis which is usually accepted as the standard procedure for assessing the performance of medical diagnostic tests, was applied to explore the changes in concentrations of endogenous metabolites potentially involved in breast cancer. So far, 5 metabolites have been identified as differentially expressed between the patients and controls Oleamide, the most significantly increased in breast cancer plasma samples, yielded an excellent classification ability for discriminating breast cancer patients from healthy controls. Although previously reported in vitro, this is the first time to our knowledge this molecule has been found increased in breast cancer patients. The combination of these markers into a single multivariate model provided an improved predictive power than markers alone. In summary, using this untargeted metabolomics approach, we have found clear metabolic alterations which might lead to the identification of new potential biomarkers in breast cancer.



**Poster #: 106**

**Abstract #: 2578**

**Abstract Title:** MetaboTools: Analysis of extracellular metabolomic data in the context of the metabolic model

**Authors:** Maike Aurich, Ronan MT Fleming, Ines Thiele,

**Presenting Author Affiliation:** Luxembourg Centre for Systems Biomedicine

**Abstract Submission:**

Extracellular metabolomic data provide a direct read-out of the intracellular metabolism, yet in silico approaches are needed to facilitate the analysis of the metabolic mechanisms giving rise to the increasingly complex data sets. We previously predicted differences in cell metabolism through integration of semi-quantitative extracellular metabolomic data into the context of a metabolic model 1).

Our methods have been compiled to form the MetaboTools, which comprise more than 20 matlab functions supporting (1) the generation of condition-specific metabolic models from both quantitative and semi-quantitative extracellular metabolomic data, (2) the prediction of intracellular metabolic phenotypes and the outcome of systems perturbation, and allows (3) multi-omics integrative analysis in the context of the metabolic model. Along with the MetaboTools we provide a protocol that describes in detail the consecutive steps to the analysis of extracellular metabolomic data in the network context. We discuss, which input data is required to integrate quantitative and semi-quantitative metabolomic data into the network context, enabling scientists to take the requirements into account already in the planning phase of their experiments. Moreover, we provide tutorials of the computational workflow. The tutorials recapitulate in a step-by-step manner the integration of the data and the prediction of the intracellular metabolism based on two distinct data sets. Hence, they provide hands-on training material on how the workflow can be adopted to different data sets, and guide through the reproduction of the results as presented in our primary work<sup>1</sup>.

Taken together, we highlight the potential of using the metabolic model in the downstream analysis and interpretation of extracellular metabolomic data. The MetaboTools together with the accompanying tutorials and the protocol will make the interpretation of extracellular metabolomic data in the model context accessible to a large scientific community.

**References:**

- 1 Aurich, M. et al., *Metabolomics* 11, 603-619, doi:10.1007/s11306-014-0721-3 (2015).

**Poster #: 107**

**Abstract #: 2011**

**Abstract Title:** Combination therapies targeting metabolic processes inhibits tumourigenesis.

**Authors:** Paola Marignani,

**Presenting Author Affiliation:**

**Abstract Submission:**

Metabolic adaption of tumour cells is necessary to meet biosynthesis and energy needs of a growing cancer. The energy sensing kinase AMPK is responsible for maintaining AMP/ATP ratio, serving as a metabolic checkpoint that is activated when phosphorylated by the LKB1, a tumour suppressor kinase. LKB1 is frequently found mutated in numerous cancers including 31% of HER2 breast cancer.

In our LKB1-/-NIC model, loss of LKB1 expression resulted in reduced tumour latency where tumours were biochemically characterized as hyperactive mTOR, along with metabolic changes characteristic of Warburg effect, namely elevated ATP, LDH, PDH expression and enhanced lactate. Based on these finding, we conducted a pre-clinical studies to evaluate novel combinatorial therapies on tumourigenesis.

We report that targeting PI3K-p70S6K pathways with competitive NVP-BEZ235 inhibitor was not as effective at reducing tumourigenesis as targeting mTOR and glycolysis with AZD8055 and 2-DG monotherapies, respectively. Interestingly, simultaneous inhibition of these pathways with AZD8055/2-DG combination was significantly more effective at reducing mitochondria function, tumour volume and burden, culminating in reduced tumourigenesis. At the molecular level, combination treatment inhibited both mTOR signalling and blocked MAPK survival signalling that is responsible for ERK-p90RSK pathway engagement. Finally, loss of LKB1 expression in cancers should be considered a marker for metabolic dysfunction given the role LKB1 plays in regulating both AMPK activity and mTOR function.

The results of our pre-clinical studies suggest that combinatorial therapy that target mTORC1/mTORC2 and metabolism in cancer, is critical for inhibiting tumour growth. Importantly, our discovery showed that the drug combination inhibited the activation of feedback loops that are drivers of resistance, namely ERK and p90RSK. We believe that simultaneous targeting of these pathways will provide the best clinical outcome for the treatment of metabolically active cancers, as well as reduce the likelihood of recurrence.

**Poster #: 108**

**Abstract #: 2048**

**Abstract Title:** High-resolution metabolic profiling unveils the differences between glio- and neuroblastoma

**Authors:** Kyoung-Soon Jang,

**Presenting Author Affiliation:** Korea Basic Science Institute

**Abstract Submission:**

Glioblastoma multiforme (GBM) is the most common and aggressive malignant primary brain tumor that represents 15% of brain tumors. GBM is also known to have a radical proliferation ability that complicates the therapeutic modulation of cancer progression.

In this study, we investigated cellular metabolites from human glioblastoma in comparison with those from human neuroblastoma (NB). Methanol extracts from human GBM and NB cell lines were comparatively analyzed using a high-resolution 15 Tesla Fourier transform ion cyclotron resonance (15 T FT-ICR) mass spectrometry to obtain high-accuracy profiles in both positive and negative ion modes. The multivariate data analyses such as principal component analysis (PCA) and partial least square-discriminant analysis (PLS-DA) were employed to differentiate key metabolites between GBM and NB cell lines. Following UPLC/ESI-Q-TOF/MS analysis was used to confirm the key metabolites. Using these metabolomics approaches, hundreds of metabolites were predicted, and more than 100 metabolites could be identified and quantified from the human GBM and NB, suggesting key metabolite factors differentiating glio- and neuroblastoma.

The distinct metabolic factors observed in GBM would lead to the development of potential targeted cancer therapy for the malignant brain tumor.

**Poster #: 109**

**Abstract #: 2399**

**Abstract Title:** Plasma metabolome associated with chronic kidney disease in general population

**Authors:** Sei Harada, Ayako Kurihara, Kota Fukai, Ayano Takeuchi, Tomonori Okamura, Akiyoshi Hirayama, Masahiro Sugimoto, Tomoyoshi Soga, Masaru Tomita, Toru Takebayashi,

**Presenting Author Affiliation:** Keio University

**Abstract Submission:**

Chronic kidney disease (CKD) is increasing and frequently leads to adverse outcomes, including death, cardiovascular events, and the development of end-stage renal disease. In both public health and clinical aspects, it is highly required to establish biomarkers which can detect CKD in early stage, and monitor disease progression and response to treatment. In this study, we examined plasma metabolites altered by kidney function, using large metabolomics profiling data of general population.

Fasting plasma samples were collected from 11,002 Japanese who participated in Tsuruoka Metabolomics Cohort Study since 2012. By the end of 2015, we had performed plasma global metabolomic profiling by CE-MS in 2,155 males (62.5±8.3 years old) and 2,634 females (62.3±8.5 years old) of participants in 2012 and 2013. To reveal CKD biomarkers in plasma, we performed PLS-DA stratified by sex, using all polar metabolite concentrations stably quantified.

168 males (8%) and 134 females (5%) had CKD defined as eGFR 1.5) in males, including phenylalanine, choline, and uric acid. This result was also confirmed in females. Then, we described ROC curves using these eight biomarkers. Area under the curve was 0.816 (95% CI: 0.774-0.860) in males, and 0.795 (95% CI: 0.751-0.842) in females. Therefore, these metabolites were considered as good biomarkers of CKD development in general population.

Interestingly, all of the significant biomarker concentrations were higher in the CKD patients' plasma. The elevation of some markers such as uric acid, damaging tissues of kidney, was considered as the cause of CKD. Other markers were considered to be elevated in plasma because excretion from kidney were decreased. To reveal the causality between CKD and metabolites, we are now conducting the follow-up survey.

**Poster #: 110**

**Abstract #: 2032**

**Abstract Title:** Metabolomics based on J-resolved NMR spectroscopy in monitoring of anticancer treatment toxicity

**Authors:** Lukasz Boguszewicz,

**Presenting Author Affiliation:** Maria Sklodowska-Curie Memorial Cancer Center and

**Abstract Submission:**

Introduction

Combined modality therapy – sequential and concurrent radiotherapy and chemotherapy, a standard organ preservation treatment method for head and neck squamous cell carcinomas (HNSCC) – results in temporary or permanent toxicity considered as changes in normal tissues and/or involved regions. We aimed to investigate molecular processes reflecting acute radiation syndrome (ARS) in HNSCC patients using <sup>1</sup>H NMR-based metabolomics of blood serum.

Methods

The studied group consisted of 45 HNSCC patients treated with radiotherapy/chemoradiotherapy (RT/CHRT). Severity of ARS was monitored throughout and after the treatment until the resolution of all the ARS symptoms.

The patients were divided into two classes (of high and low ARS) on the basis of the highest individual ARS value observed during the treatment. Blood samples were collected within a week after RT/CHRT completion. The 2D J-resolved NMR spectra of serum samples were acquired on 400.13 MHz Bruker spectrometer at 310K and their 1D projections were analyzed using principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA).

Results

Unsupervised PCA method applied to 1D projections of 2D J-resolved NMR spectra shows a clear separation between the high and low ARS cases, while the S-line plot of OPLS-DA reveals the metabolic features characteristic for high ARS: the increased signals of N-acetyl-glycoprotein (NAG) and acetate, as well as decrease of choline and the metabolites involved in energy metabolism, such as branched chain amino acids (BCAAs), alanine, creatinine, carnitine and glucose. NAG was found to be positively correlated with C-reactive protein (CRP), while alanine and BCAAs showed negative correlation with CRP. We also observed a positive correlation between acetate and a percentage-weight-loss during the treatment.

Conclusions

Metabolomics based on 1D projections of the J-resolved NMR spectra seems to be of great potential in the quest for HNSCC treatment toxicity biomarker.

Funded by the National Science Centre grant 2015/17/B/NZ5/01387

**Poster #: 111**

**Abstract #: 2058**

**Abstract Title:** 13C6-Glucose labeling metabolomics reveals effects of PTEN mutation on prostate cancer metabolism

**Authors:** Wang Zhichao, Jun Zeng, Guowang Xu, Hailong Piao,

**Presenting Author Affiliation:** DICP, CAS

**Abstract Submission:**

Metabolic reprogramming is one of the hallmarks of cancer<sup>1</sup>, but the understanding of the metabolic pathway regulation of cancer remains a challenge. Isotopic labeling-metabolic analysis is supposed to be a powerful tool to provide new insight in metabolites function by providing information about the dynamics of specialized metabolite accumulation and turnover.

PTEN mutation occurs frequently during the progression of prostate cancer<sup>2</sup>, but its regulation mechanism on metabolic network remains ambiguous. In this study, four prostate cancer cell lines with mutant PTEN (PC-3, C4-2) and normal PTEN (DU145, VCaP) were used for a time series (0h, 0.25h, 4h, 12h, 24h) 13C6-glucose labeling experiment. CE-MS based metabolomics for polar metabolomics and LC-MS based lipidomics combined with stable isotope labeling experiment were carried out to investigate the effects of PTEN mutation on prostate cancer metabolic reprogramming. Based on our in-house developed software, a comprehensive profile of “static” global response, “dynamic” labeling fraction and labeling patterns can be finally achieved for the analysis, which goes beyond the information reached by current stable isotope-assisted metabolomics approaches. Static quantities of metabolites indicated elevated metabolites in TCA such as citrate, malate, fumarate, while dynamic atomic labeling fraction indicated elevated ratio of TCA metabolites synthesized from glucose in cell lines with mutant PTEN. Besides, the differences of citrate isotopmer distribution between PTEN mutated cell lines and PTEN normal cell lines indicated the preference for using pyruvate carboxylase rather than pyruvate dehydrogenase in PTEN mutated cell lines. Combination of the static quantities of metabolites with the dynamic atomic labeling fraction have shown to be robust tools for metabolic pathway interpretation.

**References:**

1. Hanahan, D. & Weinberg, R. A. Hallmarks of Cancer: The Next Generation. *Cell* 144, 646–674 (2011).
2. Deocampo, N. D., Huang, H. & Tindall, D. J. The role of PTEN in the progression and survival of prostate cancer. *Minerva Endocrinol.* 28, 145–153 (2003).

**Poster #: 112****Abstract #: 2156****Abstract Title:** Evaluation of gas-chromatography tandem mass spectrometry system with automated TMS derivatization in analysis of plasma metabolites**Authors:** Noriyuki Ojima, Shuichi Kawana, Yumi Unno, Yukihiro Kudo, Takero Sakai, Takashi Kobayashi, Shin Nishiumi, Masaru Yoshida,**Presenting Author Affiliation:** Shimadzu Corporation**Abstract Submission:**

Gas-Chromatography Tandem Mass Spectrometry (GC-MS/MS) enables the stable measurements with high chromatographic separation and high sensitivity, so it has been widely used in the metabolomics research. In GC-MS/MS analysis, the metabolites need to be derivatized. In case of TMS derivatization, the samples should be analyzed within 24 hours after derivatization, because some TMS derivatives deteriorate 24 hours after derivatization. To improve it, we developed an Automated TMS Derivatization GC-MS/MS System. In this study, this system was evaluated for analysis of the metabolites in human plasma.

In sample preparations, an internal standard (0.5 mg/mL of 2-Isopropylmalic acid) was added to 50  $\mu$ L of pooled human plasma. The metabolites were extracted with 250  $\mu$ L of methanol/water/chloroform (2.5:1:1) mixture and then dried by SpeedVac. The lyophilized extracts were pre-treated with methoxyamine before the analysis. The analysis was performed on AOC-6000 (Shimadzu) and GCMS-TQ8040 (Shimadzu) equipped with BPX-5 capillary column (SGE). AOC-6000 automates the TMS derivatization process and injects a sample to GC-MS/MS automatically.

To evaluate the reproducibility of this system, the 7 replicate samples were analyzed. The 179 metabolites were detected in pooled human plasma. For the detected metabolites, the peak area of each ion was calculated and normalized to the peak area of 2-isopropylmalic acid as the internal standard. The RSD% of 134 metabolites were less than 20%. To validate the deterioration of TMS derivatives, the samples which passed from 0 to 24 hours after the methoximation were analyzed. The signal intensity for Lysine, Tyrosine, Kynurenine and Tryptophan was changed in the conventional system without the Automated TMS Derivatization, but their intensity was not changed in Automated TMS Derivatization GC-MS/MS System even with the lapse of time. These results demonstrate that Automated TMS Derivatization GC-MS/MS System improves the accuracy for analysis of plasma metabolites.



**Poster #: 113**

**Abstract #: 2169**

**Abstract Title:** Urinary Metabolite Risk Biomarkers of Lung Cancer: A Prospective Cohort Study

**Authors:** Majda Haznadar,

**Presenting Author Affiliation:** Laboratory of Human Carcinogenesis, NCI, NIH

**Abstract Submission:**

Lung cancer is a major health burden causing 160,000 and 1.6 million deaths annually in the United State and worldwide, respectively. However, there are no clinically utilized biomarkers that can aid in early detection of this disease. Seeking to identify stable and reproducible biomarkers in non-invasively collected biofluids, we assessed whether previously identified metabolite urinary lung cancer biomarkers, creatine riboside (CR), N-acetylneuraminic acid (NANA), cortisol sulfate (CS) and indeterminate metabolite with m/z of 561 and detected in electrospray (ESI) positive mode were elevated in the urines of subjects prior to lung cancer diagnosis in a well-characterized prospective Southern Community Cohort Study (SCCS). Mass spectrometry was performed on a Waters XEVO G2 ESI QTOF mass spectrometer operating in ESI positive (monitoring CR and 561+) and negative (monitoring NANA and CS) modes. Urine was examined from 178 patients and 351 non-diseased controls, confirming that one of four metabolites was associated with lung cancer risk in the overall case-control set, whereas two metabolites were associated with lung cancer risk in European-Americans. Odds ratio of lung cancer associated with elevated CR levels, and adjusted for smoking and other potential confounders, was 2.0 (95%CI 1.2-3.4P=0.01). In European-Americans, both CR and NANA were significantly associated with lung cancer risk (OR=5.3 (95%CI 1.6-17.6 P=0.006 and OR=3.5 (95%CI 1.5, 8.4P=0.004), respectively). However, race itself did not significantly modify the associations. Receiver Operating Characteristic (ROC) analysis showed that adding CR and NANA to a model containing previously established lung cancer risk factors led to a significantly improved classifier (P=0.01). Increasing urinary levels of CR and NANA displayed a positive association with increasing tumor size, strengthening a previously established link to altered tumor metabolism. These replicated results provide evidence that identified urinary metabolite biomarkers have a potential utility as non-invasive, clinical screening tools for early diagnosis of lung cancer.

**Poster #: 114**

**Abstract #: 2239**

**Abstract Title:** The external and intra-laboratory validation of potential biomarker candidates for prostate cancer

**Authors:** Renata Bujak, Wiktoria Struck-Lewicka, Malgorzata Patejko, Marta Kordalewska, Tomáš Kovalczuk, Agnieszka Ulanowska, Grzegorz Straczynski, Roman Kaliszan, Michal Markuszewski,

**Presenting Author Affiliation:** Medical University of Gdansk

**Abstract Submission:**

Prostate cancer (CaP) constitutes a leading cause of cancer deaths in men worldwide [1]. The current diagnostic test still lacks specificity. The aim of this study was to perform external and intra-laboratory validation of previously reported metabolic CaP markers [2]. Urine samples from CaP patients (n=39) and healthy volunteers (n=43) were analyzed by both gas chromatography triple quadrupole mass spectrometry (GC-QqQ/MS) and comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (GCxGC-TOF MS, Pegasus 4D system). Data deconvolution, alignment and normalization were performed using ChromaTOFTM and AMDIS software. Statistical analyses were conducted with the use of both Statistical Compare feature in ChromaTOFTM and SIMCA P+ software. Principal component analysis (PCA) was applied to detect potential outlier and confirm quality of analysis. To select metabolites as those contributing the most into group classification orthogonal partial least squares-discriminant analysis (OPLS-DA) was used. Aconitic acid, alanine, threonic acid and meso-erythritol could be assigned as validated potential CaP biomarker candidates. This study may be considered as an external intra-laboratory validation in non-targeted metabolomics research. It should be underlined that these results should be confirmed using qualitative targeted metabolomics approach based on larger set of samples to select diagnostic CaP markers.

**Poster #: 115**

**Abstract #: 2242**

**Abstract Title:** Urine metabolic fingerprinting in renal cell carcinoma for proper classification of cancer patients and healthy volunteers

**Authors:** Marta Kordalewska, Renata Bujak, Karolina Siedlecka, Wiktoria Struck-Lewicka, Arlette Yumba Mpanga, Marcin Markuszewski, Marcin Matuszewski, Roman Kaliszan, Michal Markuszewski,

**Presenting Author Affiliation:** Department of Biopharmaceutics and Pharmacodynamic

**Abstract Submission:**

Renal cell carcinoma (RCC) is one of the 10 most common cancer types. Due to the lack of specific symptoms and diagnostic methods, RCC is frequently misdiagnosed and detected as another abdominal disease. Therefore, development and application of new high-throughput and specific diagnostic methods is essential for early detection of RCC. In the present study urine metabolic fingerprinting was performed for understanding and explanation of RCC pathomechanisms.

Urine samples collected from RCC patients and healthy volunteers were analyzed with the use of HPLC-TOF/MS in positive and negative ionization modes, as well as GC-QqQ/MS in scan mode. The obtained datasets were processed using deconvolution, alignment, normalization and filtration steps. Afterwards, uni- and multivariate statistical analyses were performed. Statistically significant metabolites were selected according to adjusted p value (FDR p value 1. The identification of selected metabolites using NIST, HMDB, METLIN, KEGG and CEU Mass Mediator databases allowed for creation of a list of putative markers and related biochemical pathways which they are involved in.

Selected altered metabolites were found to be involved in amino acid, purine, lipid and glucose metabolism as well as TCA cycle.

The obtained results suggest that urine metabolic fingerprinting is a powerful tool which might be useful in research for RCC diagnosis and eventual further explanation of its molecular pathomechanisms.

**Poster #: 116**

**Abstract #: 2306**

**Abstract Title:** Metabolic alterations and the effect of sophocarpine and matrine therapy in an experimental cancer cachexia murine model

**Authors:** xinru liu,

**Presenting Author Affiliation:** second military medical university

**Abstract Submission:**

Cancer cachexia is a metabolic phenotype disorder characterized by loss of skeletal muscle and adipose tissue. Approximately 50-80% of cancer patients develop cachexia, and 20% of cancer deaths attributable to cachexia. Cachexia indicates poor prognosis and is associated with physical function damage, a reduction in response to therapy, a negative impact on quality of life and survival expectancy. The pathogenesis of cancer cachexia is complex. Multiple biologic metabolic pathways are involved, including glucose metabolism, protein metabolism and muscle depletion, lipid metabolism, and energy metabolism. Several blood biomarkers have been identified for cancer cachectic diagnosis, such as IL-1, IL-6, and TNF- $\alpha$ , Ghrelin, myoglobin, CRP, and UCP1. All these biomarkers are linked to systemic inflammation, since the inflammatory response is the main driving force behind the metabolic changes in cancer.

Currently, different therapeutic approaches are available. For instance, administration of omega-3 fatty acids, ghrelin, or actRIIB. Although a significantly increased number of studies focuses on cancer cachexia over the past 10 years, the biology and molecular mechanisms are still not fully understood, effective biomarkers in blood/urine are far from being universal and applicable. Recently, it was reported that sophocarpine and matrine exhibit potentially beneficial biological and pharmacological effects such as anti-inflammation, anti-bacterium, antiviral, immunity-regulation, and anti-tumor activities. Our colleagues' studies have reported significant anti-inflammation and anti-cachectic effects of sophocarpine and matrine in colon26 adenocarcinoma-bearing mice.

Metabolomics, the global study of the unique chemical fingerprints that specific cellular processes leave behind, has been used to understand cancer cachexia systematically. In the present study, we identified 157 potential biomarkers in cachectic murine serum samples, built up a metabolic pathways network connecting 138 related identified metabolites, and focused on biomarkers was regulated by natural compounds using nontargeted biochemical profiling and bioinformatics.

**Poster #: 117**

**Abstract #: 2546**

**Abstract Title:** Biomarker discovery using high resolution metabolomics of Korean male lung cancer patient serum in association with alcohol consumption

**Authors:** Youngja Park, Aryo Pamungkas

**Presenting Author Affiliation:** Korea Univeristy

**Abstract Submission:**

As the mortality rate of lung cancer increases, deeper understanding is necessary to explore risk factors that may lead to this malignancy, especially regarding the lifestyle-related risk factors. Also, current diagnostic technologies still fall short in detecting the early stages of this disease leading to late diagnosis. Thus, the need to explore new biomarkers to aid the detection of lung cancer is important. In this regard, this study aims to apply high resolution metabolomics (HRM) using LC-MS to detect significant compounds that might contribute in inducing lung cancer and find the correlation of these compounds to the subjects' alcohol consumption history. All subjects were currently non-smokers. Comparison was done between healthy control (with no alcohol consumption history) and lung cancer patients (with alcohol consumption history) for metabolic differences. The univariate analysis was performed, including a false discovery rate (FDR) of  $q=0.05$ , to determine the significant metabolites between the analyses. Hierarchical clustering analysis (HCA) was done to discriminate metabolites between the control and case subjects. Selected compounds based on significant features of human serum then experienced MS/MS examination, showing that for many  $m/z$ , the patterns of ion dissociation matched with standards. Then, the significant metabolites were identified using Metlin database and features were mapped on the human metabolic pathway mapping tool of the Kyoto Encyclopedia of Genes and Genomes (KEGG). Using metabolomics-wide association studies, metabolic changes were observed among control group and lung cancer patients. Four potential biomarkers, retinol (287.23,  $[M+H]^+$ ), cholecalciferol (385.34,  $[M+H]^+$ ), thiamin triphosphate (505.01,  $[M+H]^+$ ) and L-proline (116.07,  $[M+H]^+$ ) were among the significant compounds found to have contributed in the discrimination between these groups, suggesting that these compounds might be related in the development of lung cancer in association with alcohol consumption.

**Poster #: 118**

**Abstract #: 2258**

**Abstract Title:** Metabolomic analysis of Nicotinamide phosphoribosyltransferase (NAMPT) inhibition on human cancer cell line

**Authors:** XUEJIAO YIN,

**Presenting Author Affiliation:** National University of Singapore

**Abstract Submission:**

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.

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. In order to study the quick response to FK866 and GMX1778 in cancer cell lines, we studied a lung cancer cell type A549. Cells were grown in 6-well plate: (1) control (C), DMSO (2) FK866 1000nM (F). After the cells were grown to 80-90% confluency, the four different conditions were added to medium and incubate for 24 hours before harvest.

We found that adenine, adenosine, adenosine monophosphate (AMP), were up-regulated as compared to control group, indicating NAMPT inhibition significantly alters purine biosynthesis. This particular increase was probably due to the inhibition of AMP dehydrogenase (AMPDH) resulting from NAD depletion as this enzyme requires NAD for activity. We observed down-regulation NAD and Nicotinamide, which can be explained with the suppression of NAD biosynthesis using FK866 drug. Choline, Phosphocholine (PC), triglycerides, Phosphoethanolamine (PE) and Sphingomyelin (SM) were observed to be up-regulated, which suggests there is cell-specific response of lipid metabolism to NAMPT inhibition with FK866.

**Poster #: 119**

**Abstract #: 2499**

**Abstract Title:** Effects of anti-CD44 Monoclonal Antibodies on Metabolic Profiles of Acute Myeloid Leukemia

**Authors:** Nour Madhoun, Samah Gadhoum, Abdul-Hamid Emwas, Jasmeen Merzaban,

**Presenting Author Affiliation:** King Abdullah University of Science and Technology

**Abstract Submission:**

Acute myeloid leukemia (AML) is a heterogeneous disease characterized by accumulation of immature hematopoietic cells due to a blockage in myeloid differentiation at various stages. Previous experiments have shown that ligation of CD44, a cell surface glycoprotein strongly expressed on all AML cells, with anti-CD44 monoclonal antibodies (mAbs) could reverse the differentiation blockage of leukemic blasts and inhibit their proliferation in most AML subtypes. Thus rendering CD44 a promising target for AML therapy. However, the underlying molecular mechanisms of the induction of differentiation by anti-CD44 mAbs have not been fully elucidated. In this study, we are interested in determining the metabolic changes that take place during the treatment of AML cells with the CD44-specific mAb. The state-of-the-art Nuclear Magnetic Resonance (NMR) technology was used to identify the metabolic profile for HL60 cells and monitor the overall metabolic consequences of treatment with anti-CD44 mAbs.

<sup>1</sup>H-NMR experiments demonstrated that anti-CD44 treatment induced considerable changes in the metabolic profiles of HL60 cell lines. A total of 23 identified metabolites were classified as statistically significant ( $p < 0.05$ ). Loading plots for principal component analysis revealed increase in the levels of isoleucine, valine, leucine, alanine, acetate, glutamine, succinate, myo-inositol, glycine, formate, phenylalanine, while remarkable decrease in glycerophosphocholine, phosphocholine, choline, aspartate, fumarate, malonate, and creatine were observed. These most notable responses included changes to tricarboxylic acid cycle (TCA) intermediates, including succinate and fumarate. Moreover, This was validated by dramatically reduction in succinate dehydrogenase enzyme (SDH) activity in HL60 by the treatment of anti-CD44 mAbs.

Our findings demonstrate that metabolite profiles describes the actual functional state of the cells and opens new perspectives to use metabolic profiling as a tool to support the potential possibilities for the development of CD44-targeted therapy of AML.

**Poster #: 120**

**Abstract #: 2517**

**Abstract Title:** Metabolite Profiling of Brain Tumor Cells under different nutritional conditions using GC-MS

**Authors:** Henry Oppermann, Jeevan Pharma, Mandy Berndt-Paetz, Frank Gaunitz, Claudia Birkemeyer,

**Presenting Author Affiliation:** University Hospital Leipzig Neurosurgery

**Abstract Submission:**

Tumor cell growth is highly dependent on glucose which is thought to be preferably metabolized to lactate. Although glycolytic ATP production is important for tumor cell survival, several biochemical pathways used for the production of essential cellular building blocks during proliferation such as proteins, membranes and DNA can be fed by glycolytic intermediates. In order to understand how the availability of nutritional glucose contributes to the synthesis of such cellular building blocks in tumor cells we performed a metabolic profiling with glioblastoma brain tumor cells that were starved for 20 hours and then refeed with medium containing different concentrations of glucose. After 24 hours of refeeding the metabolic profile was determined using GC-MS.

Among the different metabolites that could be synthesized from glucose we found glycolytic intermediates such as glucose-6-phosphate or fructose-6-phosphate as well as side products from glycolytic activity such as sorbitol or ribose-5-phosphate and downstream products such as pyruvate, lactate and alanine correlating with the applied glucose concentration. In addition, the production of methylglyoxal, the toxic byproduct of glycolysis, directly correlated with the glucose concentration supplied confirming the notion that it is a marker of glycolytic activity alongside with extracellular lactate. Interestingly, we also observed a significant production of metabolites such as citrate, succinate, alpha-ketoglutarate or glutamate that are generally synthesized by the activity of enzymes belonging to the tricarboxylic acid (TCA) cycle and which require the synthesis of Acetyl-CoA from glycolytically produced pyruvate.

Our observation confirms that tumor cells produce several intermediates from glucose which contribute to the synthesis of building blocks in order to build up biomass, instead of the mere secretion of glycolytically produced lactate. Furthermore our results suggest that the synthesis of building blocks from glucose in these tumor cells involves also biochemical pathways other than glycolysis but belonging to the TCA cycle instead.



**Poster #: 121**

**Abstract #: 2592**

**Abstract Title:** Rapid Evaporative Ionisation Mass Spectrometry of Human Faecal Samples

**Authors:** James Alexander, Louise Gildea, Simon Cameron, James McKenzie, Frances Bolt, Nicole Strittmatter, Adele Savage, Julian Teare, James Kinross, Zoltan Takats,

**Presenting Author Affiliation:** Imperial College London

**Abstract Submission:**

Rapid Evaporative Ionisation Mass Spectrometry of Human Faecal Samples

James Alexander, Louise Gildea, Simon Cameron, James McKenzie, Frances Bolt, Nicole Strittmatter, James Kinross, Julian Teare and Zoltan Takats\*

Imperial College London

Introduction

Rapid Evaporative Ionisation Mass Spectrometry (REIMS) produces highly specific lipidomic based profiles of human tissues and microorganisms. In this first in human pilot study we developed a method for REIMS analysis of faeces and assessed its ability to identify metabolites of importance in gastrointestinal disease.

Methods

Stool samples were collected from ten patients who had undergone surgery for colorectal cancer at St Mary's Hospital, London UK. No sample preparation was required. Ex-vivo analysis was performed on stools using a modified electrosurgical handpiece and bipolar forceps with a generator (Valleylab<sup>TM</sup>) over a series of power settings to determine optimal sampling conditions. The electrosurgical aerosol was transferred in real time to a Synapt G2-S mass spectrometer (Waters Corporation) for spectral analysis. A Venturi pump system and a direct aerosol introduction method were tested. Spectra were analysed and putative metabolite identifications were made using databases. Spectra were then examined for peaks that corresponded to a library of bacterial taxon-specific lipids to assess the technique's potential for the identification of constituents of the gut microbiota.

Results

Faecal REIMS method optimisation demonstrated that a modified electrosurgical handpiece with introduction of aerosol to the mass spectrometer provides highly reproducible spectra with good signal to noise ratio. The stool lipidome identified by REIMS shows glycerolipid and glycerophospholipid species. Tentative bacterial taxonomic biomarkers were identified for Helicobacteriaceae, Bacteroidetes and Burholderiaceae.

Conclusion

REIMS analysis of faeces delivers immediate and robust spectral data encompassing metabolites of known importance in digestive disease. Furthermore it is possible to identify taxon-specific bacterial lipids, suggesting that this technique could be used for rapid analysis of gastrointestinal pathogens and the gut microbiome.

**Poster #: 122**

**Abstract #: 2019**

**Abstract Title:** Metabonomic changes in pancreatic tissues from intraepithelial neoplasia to ductal adenocarcinoma

**Authors:** Jianghua Feng,

**Presenting Author Affiliation:**

**Abstract Submission:**

**Introduction:** Pancreatic ductal adenocarcinoma (PDAC) is one of the most malignant tumors with an extremely poor prognosis, and its diagnosis in early phase is quite difficult but to be vitally necessary. This study was aimed at obtaining the metabolic profiles and characteristic metabolites of pancreatic intraepithelial neoplasia (PanIN) and PDAC tissues from Sprague-Dawley (SD) rats to establish metabonomic methods which served to PDAC's early diagnosis.

**Methods:** In the present study, the animal models were established by embedding 7,12-dimethylbenzanthracene (DMBA) in pancreas of SD rats to obtain PanIN and PDAC tissues. After the preprocessing of tissues, <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy combined with multivariate statistical analysis was applied to identify the potential metabolic signatures of PDAC and PanIN and their corresponding metabolic pathways.

**Results:** As results, pattern recognition models were successfully established and characteristic metabolites including glucose, amino acids, carboxylic acids and coenzymes were screened out corresponding to PDAC or PanIN. Compared with the control, PanIN and PDAC rats demonstrated some similar variation trends in some metabolites, however, kynurenate and methionine elevated in PanIN but decreased in PDAC, indicating to be specific metabolites distinguishing PanIN from PDAC. These results suggested that PanIN and PDAC share some biochemical mechanisms but some specific metabolic changes could be observed at the early of PDAC (here is PanIN), which could serve to the earlier diagnosis of PDAC.

**Conclusion:** Our results highlight the potential utility of NMR-based metabonomic strategy as promising approach in physiopathologic metabolism investigation and earlier diagnosis in PDAC patients.

**Poster #: 123**

**Abstract #: 2167**

**Abstract Title:** Multiplatform Plasma Fingerprinting in Cancer Cachexia: A Pilot Study

**Authors:** Mónica Cala, Jose A. Lopez-Martin, M. Teresa Agullo Ortuno, Carolina Gonzalez-Riano, Antonia Garcia, Coral Barbas,

**Presenting Author Affiliation:** Los Andes University

**Abstract Submission:**

Cachexia is a metabolic syndrome that affects a large amount of cancer patients, especially in advanced stages of the disease. This disorder may affect up to 80% of patients with advanced cancer and it is indirectly responsible for at least 20% of all cancer patients' deaths. Understanding cancer cachexia is a frequent unmet medical need. Its molecular basis has not been extensively studied in humans, and the physiopathology remains poorly understood. In the present study, a pilot multiplatform metabolomics approach was used to obtain a global picture of metabolic alternations that occur in cancer cachexia. Study was performed on plasma samples obtained from 15 cancer (ca) patients (pts), distributed as follows: Cachexia (CX): 8 pts (pancreatic ca: 3, melanoma: 3, biliary duct ca: 2) control (CN): 7 pts (colon ca: 1, esophageal ca: 1, gastric ca: 2, pancreatic ca: 1, melanoma: 1, sarcoma:1). Samples were analyzed by the three platforms commonly used in metabolomics studies (GC-MS, LC-MS and CE-MS), to increase metabolite coverage. Differences between profiles from CX and CN groups, obtained within each technique, were evaluated with univariate (UVA) and multivariate (MVA) analysis using a nonparametric t test, principal component analysis (PCA) and orthogonal partial least squares regression (OPLS-DA). The metabolite with highest increase was cortisol (fold change 1.67,  $p = 0.03$ ). The largest affected group of metabolites was amino acids and derivatives, all decreased. Glycerophospholipids, sphingolipids, carboxylic acids and derivatives, and indoles were also decreased. Despite tumour type heterogeneity and the small sample size of this study, cancer-related cachexia is significantly associated with a metabolomic signature that represents an increased catabolism. Of note, the increased values of cortisol should lead us to revisit the use of glucocorticoids in this setting. Substitutive therapy for some of the observed deficiencies might deserve clinical exploration.

**Poster #: 124**

**Abstract #: 2257**

**Abstract Title:** Utilizing large scale metabolomics profiling to identify patients with high risk smoldering myeloma

**Authors:** Wilson Gonsalves, Tumpa Dutta, Shaji Kumar, Taro Hitosugi, S. Vincent Rajkumar, K. Sreekumaran Nair,

**Presenting Author Affiliation:** Mayo Clinic

**Abstract Submission:**

Multiple myeloma is a devastating hematological malignancy of clonal plasma cells in the bone marrow that is associated with either anemia, hypercalcemia, destructive lytic bone disease or renal failure. Smoldering multiple myeloma is comprised of patients with clonal plasma cells in the bone marrow but no evidence of end organ damage as seen in multiple myeloma. They may progress to myeloma at quickly (high risk) or remain indolent for a long time (standard risk). At this time, it is not possible to distinguish between standard and high risk smoldering myeloma. We identified 25 patients with smoldering myeloma who had peripheral blood and bone marrow samples stored in the biobank. Of these patients, 15 patients progressed to multiple myeloma within 24 months and were considered to have high risk disease and the remaining 10 patients either did not progress or progressed to myeloma only after 55 months of follow up. LC-MS untargeted metabolomics profiling was utilized in 25 peripheral blood plasma samples, 25 bone marrow plasma samples and 25 bone marrow derived clonal plasma cells samples to distinguish between the standard and high risk smoldering myeloma patients. There were 42 metabolites differentially expressed between the bone marrow derived clonal plasma cells samples of standard and high risk smoldering myeloma patients. There were 80 metabolites differentially expressed between the peripheral blood plasma samples of standard and high risk smoldering myeloma patients. There were 52 metabolites differentially expressed between the bone marrow plasma samples of standard and high risk smoldering myeloma patients. PCA plot analysis was able to distinguish between the standard and high risk smoldering myeloma patients. Future studies are planned to validate the utility of accurately identifying patients with high risk smoldering myeloma as they may likely benefit from early therapy prior to developing end organ damage.

**Poster #: 125**

**Abstract #: 2278**

**Abstract Title:** Lung Cancer Associated Volatile Organic Compounds Detection Using a Novel Portable Gas Chromatographic Device Integrated MEMS and CMOS Technology

**Authors:** San-Yuan Wang, Yufeng Tseng, Te-Hsuen Tzeng, Chun-Yen Kuo, Po-Kai Huang, Yen-Ming Huang, Wei-Che Hsieh, Yu-Jie Huang, Po-Hung Kuo, Shih-An Yu, Si-Chen Lee, Wei-Cheng Tian, Shey-Shi Lu,

**Presenting Author Affiliation:** National Taiwan University

**Abstract Submission:**

The average life is getting longer with the advances in medical technology. However, the world population is aging gradually and most of the elders have some chronic diseases, such as lung cancer. In order to discover the disease earlier, monitoring their physiological parameters rapidly at home is one of the solutions. One of the non-invasive ways to diagnose the elders who may suffer from lung cancer is to analysis their exhaled air.

To analysis the elders' exhaled air at home, a small size, easy to use, portable, and low cost system is necessary. Hence, a portable micro gas chromatographic device integrated with micro-electro-mechanical systems (MEMS) and complementary metal-oxide-semiconductor (CMOS) technology is developed to detect the volatile organic compounds from the exhaled air. This device is integrated with a MEMS pre-concentrator, a MEMS separation column, and a CMOS system-on-chip. The chromatographic peaks are detected using continuous wavelet transform and the overlap peaks are deconvoluted using particle swarm optimization.

The experimental results show that this device is able to detect seven types of lung cancer associated volatile organic compounds including acetone, 2-butanone, benzene, heptane, toluene, m-xylene, and 1,3,5-trimethylbenzene. The concentration linearity is  $R^2=0.985$  and the detection sensitivity is up to 15ppb with 1,3,5-trimethylbenzene.

**Poster #: 126**

**Abstract #: 2290**

**Abstract Title:** Iso2Flux: A new software for  $^{13}\text{C}$  fluxomics developed in the framework of PheNomeNal

**Authors:** Marta Cascante Serratosa, Carles Foguet, Pedro de Atauri, Vitaly Selivanov,

**Presenting Author Affiliation:** Faculty of Biology, Department of Biochemistry and

**Abstract Submission:**

We present Iso2Flux a new software capable of  $^{13}\text{C}$  assisted fluxomics. Iso2Flux is capable of integrating a wide array of experimental measurements ( $^{13}\text{C}$  data, metabolite consumptions and productions and gene expression data) to identify the steady state metabolic flux distribution that is most consistent with experimental measurements. The software is developed in python and is based on the COBRApy package allowing seamless integration with constraint based models. Iso2Flux has been optimized for maximal performance thanks to the automatic conversion of the isotopomer propagation model to an EMU (Elementary Metabolite Units) model and the compilation of the generated model into a C module. Moreover, Iso2Flux has been designed as a flexible tool that can be operated both through command line and graphical user interface. Additionally, Iso2Flux will be integrated in the workflow for fluxomics of the PheNomeNal project (EC-654241) which aims to build an e-infrastructure for metabolomics data.

**Poster #: 127**

**Abstract #: 2292**

**Abstract Title:**  $^{13}\text{C}$  Metabolic Flux Analysis of MCF7 cells with impaired mitochondria

**Authors:** Ibrahim Halil Polat, Vitaly Selivanov, Miriam Tarrado-Castellarnau, Silvia Marin, Marta Cascante Serratos,

**Presenting Author Affiliation:** Faculty of Biology, Department of Biochemistry and

**Abstract Submission:**

Mitochondria are cellular organelles involved not only in production of ATP and several intermediates for the synthesis of biomolecules, but also in various cellular processes including regulation of metabolic flux and programmed cell death (apoptosis). It was previously believed that the enhanced glycolysis in cancer cells (Warburg effect) was due to nonfunctional mitochondria of those cells however, recent studies revealed that cancer cells with depleted mitochondrial DNA show reduced tumorigenic potential both in vitro and in vivo. Metabolic flux analysis of cancer cells is a competent tool to be used in order to understand the regulation of cancer cell metabolism and point out new therapeutic strategies. In this perspective, the analysis of the isotopologue distribution resulting from incubation with stable isotope tracers using the in-house developed software Isodyn, has been used to assess the metabolic flux profile of human breast adenocarcinoma cells MCF7 with impaired mitochondria. Spectrophotometric measurements and mass isotopomer distribution analysis (MIDA) have revealed that mitochondrial impairment with oligomycin leads to a metabolic reprogramming on various pathways including glycolysis, pentose phosphate pathway, TCA cycle and amino acid incorporations. More quantitative and specific results derived by metabolic flux profile obtained by Isodyn are also concordant with the changes determined in these pathways. Significant changes on the fluxes of certain key enzymes of the central carbon metabolism of MCF7 cells with impaired mitochondria has pointed out promising targets on cancer treatment.

The study now is now going on with specific inhibitions of the specified pathways in order to further validate the effects of the impairment of mitochondria and discover the mechanisms lying behind.



**Poster #: 128****Abstract #: 2379**

**Abstract Title:** Amino acids profiling as biomarkers to identify healthy individuals: a targeted metabolomic approach

**Authors:** Alessandro Silva, Josiane Steluti, Aline Carvalho, Andreia Miranda, Antonio Augusto Carioca, Ismael da Silva, Regina Fisberg, Dirce Marchioni,

**Presenting Author Affiliation:** University of São Paulo

**Abstract Submission:**

**Background.** Obesity is a metabolic disorder, characterized by the increase of body mass index (BMI>30kg/m<sup>2</sup>), that compromises the physiological functions and triggers severe morbidities for humans. Therefore, would be valuable to employ novel tools, like metabolome profiling, to investigate metabolic characteristics in metabolically healthy people. **Objective.** This study aimed to identify the amino acids (AA) as biomarkers to distinguish metabolically healthy (MHI) from unhealthy (MUI) individuals. **Methods.** The blood sample was obtained from 169 adults (≥20 years) who participated in the population based cross-sectional study “Health Survey of Sao Paulo” (ISA-Capital/2008). The targeted AA metabolome profiling were analyzed by HPLC/MS methods. MUI was characterized as having more than 3 of health abnormalities parameters: medicine use, fasting glucose, triglycerides, HOMA-IR, insulin, cholesterol, adiponectin, leptin, and BMI. Hypothesis tests were used to evaluate the difference between MHI and MUI according to their biochemical parameters. Principal Component Factor Analysis (PCFA) with varimax orthogonal rotation was performed to generate the AA profiles. It was assessed the association between AA profiles and MHI, adjusted by age and sex. Multiple logistic regression analysis was applied to evaluate the possibility of AA profiles to predict MHI, independent of age and sex. **Results.** No significant difference between the metabolic healthy and unhealthy obese was observed, considering the biochemistry parameters. The PCFA produced 4 profiles (P): P1 (Ile, Leu, Lys, Phe, Trp, Tyr, Val), P2 (Arg, Asn, Gln, Glu, His, Met, Ser, Thr), P3 (Cit, Gly, Orn) and P4 (Ala, Asp, Pro). P1 ( $\beta=0.59$ ,  $p=0.000$ ), P2 ( $\beta=-0.37$ ,  $p=0.016$ ), P3 ( $\beta=-0.37$ ,  $p=0.015$ ) revealed significant association with the MUI. P1 (OR=2.20 IC95%: 1.43-3.39) was the one which increased the chance to predict MUI significantly. **Conclusion.** The AA profiling was related to MUI independent of age and sex and could indicate the chance of individuals to have unhealthy metabolism.

**Poster #: 129**

**Abstract #: 2322**

**Abstract Title:** The Landscape of Metabolic Changes Across Tumor Types

**Authors:** Ed Reznik, Augustin Luna, Arman Aksoy, Eric Minwei Liu, Chris Sander,

**Presenting Author Affiliation:** Memorial Sloan-Kettering Cancer Center

**Abstract Submission:**

In this work, we assembled a cross-cancer collection of metabolic profiles on approximately 900 (~400 normal and ~500 tumor) samples from 11 publicly available studies of 7 cancer types. We discuss the challenges associated with data normalization and the common mapping of metabolic identifiers across different studies of the collected data. We complete a meta-analysis of the data, examining the metabolic variation associated with the transformation of normal tissues to cancer. Our findings reveal that the extent to which tumors metabolically differ from normal tissues is highly dependent on tissue of origin, and we find recurrent metabolic alterations that are associated both with central carbon metabolism, as well as less-studied pathways. Lastly, we identify metabolites whose levels are associated with tumor progression by integrating clinical information (e.g. tumor stage and grade), highlighting the role that metabolism plays in the development of aggressive features in malignancies. We believe that this work will serve as a benchmark to future meta-analyses of metabolic profiling from large-scale cancer studies.

**Poster #: 130****Abstract #: 2116**

**Abstract Title:** A novel plasma based assay for the differentiation of pancreatic cancer from chronic pancreatitis

**Authors:** Beate Kamlage, Julia Mayerle, Holger Kalthoff, Regina Reszka, Erik Peter, Sandra González Maldonado, Susan Carvalho, Christian Pilarsky, Philipp Schatz, Robert Grützmann, Markus Lerch,

**Presenting Author Affiliation:** metanomics GmbH, Berlin

**Abstract Submission:**

**Introduction:** Pancreatic ductal adenocarcinoma carcinoma (PDAC) is characterized by poor prognosis with overall 5-year survival rates of less than 5%. In resectable pancreatic cancer cases, the 5-year survival rates increase to 18-24%. Therefore, an early diagnosis is key of a potentially curative treatment and screening of patients at risk is desirable. Chronic pancreatitis (CP) has a 26-fold higher risk for the development of pancreatic cancer. Established diagnostic methods such as transabdominal ultrasound and CA19-9 testing suffer from insufficient clinical performance. Therefore, the early and preferred blood testing based differential diagnosis of the both diseases remains a clinical challenge.

**Methods:** For a case-control study in three tertiary referral centers 914 subjects were recruited with either PDAC (n=271, 135 thereof in resectable stages IA-IIB), CP (n=282), liver cirrhosis (n=100), or healthy as well as non-pancreatic-disease controls (n=261). Samples and data were subsequently analyzed within an initial exploratory study (n=201), a training study (n=474) and a test study (n=239). Fifty-two plasma samples from diabetic patients were analyzed from an independent study.

Metabolomics data was generated from plasma and serum samples by gas-chromatography–mass spectrometry and liquid-chromatography-tandem mass spectrometry (LC-MS/MS). A targeted quantitative assay (MxP® Pancreas Score) was developed that simultaneously quantifies polar and lipid metabolites after extraction and dansylation of samples by LC-MS/MS analysis.

**Results:** Data from MxP® Pancreas Score and additionally CA19-9 were analyzed by an elastic net algorithm and distinguished PDAC from CP with an area under the curve (AUC) of 0.92, resectable PDAC from CP with an AUC of 0.91, and PDAC from diabetic patients with an AUC of 0.93.

**Conclusion:** The new test has the potential to be further promoted into a laboratory developed test or an IVD assay which could greatly aid physicians in early diagnosis and treatment.

**Poster #: 131**

**Abstract #: 2195**

**Abstract Title:** Serum metabolites linked to future glioblastoma development

**Authors:** Benny Björkblom, Beatrice Melin, Henrik Antti,

**Presenting Author Affiliation:** Department of Chemistry, Umeå University

**Abstract Submission:**

Glioblastoma is associated with poor prognosis with a median survival of only one year. High doses of ionizing radiation is still the only established exogenous risk factor. To explore new potential biological risk factors for glioblastoma, we investigated alterations in metabolite concentrations in serum of healthy blood donors, diagnosed with glioblastoma up to 22 years after serum collection.

Initially we screened serum samples from a nested case-control cohort consisting of 110 future cases and 110 matched controls, using two-dimensional GCxGC-TOFMS. In total 432 unique metabolites were quantified out of which 180 were identified with high confidence. The study points out a latent biomarker for future glioblastoma consisting of nine metabolites (gamma-tocopherol, alpha-tocopherol, erythritol, erythronic acid, myo-inositol, cysteine, 2-keto-L-gluconic acid, hypoxanthine and xanthine) mainly involved in antioxidant metabolism. We specifically detect significantly higher serum concentrations of alpha-tocopherol ( $p=0.0018$ ) and gamma-tocopherol ( $p=0.0009$ ) in future glioblastoma cases. We analyzed a second nested case-control longitudinal cohort consisting of 64 future cases and 64 matched controls to verify the initial findings. In this cohort, the availability of repeated samples over time enabled detection of additional metabolites that were specifically altered in relation to time to disease onset. The latest data from our exploratory metabolomics studies, and the finding that a panel of molecules with antioxidant properties can be linked to future glioblastoma development, will be presented and discussed at the meeting.

**Poster #: 132**

**Abstract #: 2235**

**Abstract Title:** Metabolomic analysis of gastric cancer progression within the Correa's cascade using UPLC-MS

**Authors:** Guillermo Quintas, Daniel Sanjuan, Julia Kuligowski, Maria Vázquez, Anna Brunet, Carles Pericay, Maria Jose Ramírez, Sergio Lario, Lourdes Gombau, Félix Junquera, Xavier Calvet,

**Presenting Author Affiliation:** Leitat Technological Center

**Abstract Submission:**

Gastric cancer (GC) is among the most common cancers worldwide. Gastric carcinogenesis is a multistep and multifactorial process beginning with chronic gastritis induced by *Helicobacter pylori* (H. pylori) infection. This process can be described via a sequence of events known as Correa's cascade, a stepwise progression from non-active gastritis, chronic active gastritis, precursor lesions of gastric cancer (atrophy, intestinal metaplasia, dysplasia) and finally adenocarcinoma. The objective of this study was to identify a plasma metabolic pattern characteristic of GC through disease progression within the Correa's cascade. This study involved the analysis by UPLC-ESI(+)-TOFMS of plasma samples collected from 143 patients classified in four groups: without H. pylori infection H. pylori infected patients with chronic active gastritis infected or non-infected patients with precursor lesions of gastric cancer and GC. The multi-class problem was analysed through six independent partial least squares – discriminant analysis binary models implemented in a decision directed acyclic graph. PLSDA figures of merit used to evaluate the between-classes discrimination at each DDAG node were estimated by cross model validation and their statistical significance was estimated by permutation testing. Results obtained by allowed the identification of tryptophan and kynurenine as discriminant metabolites that could be attributed to indoleamine-2,3-dioxygenase (IDO) up-regulation in cancer patients leading to tryptophan depletion and kynurenine metabolites generation. Furthermore, phenylacetylglutamine was also classified as a discriminant metabolite. Our data suggest the use of tryptophan, kynurenine and phenylacetylglutamine as potential GC biomarkers. To test whether the observed differences were affected by H. pylori infection metabolomic profiles of patients with precursor lesions of gastric cancer with or without H. pylori infection were compared. Result indicated that, for this particular data set the H. pylori infection did not have a significant effect on the GC-discriminant metabolomic profile.

**Poster #: 133**

**Abstract #: 2340**

**Abstract Title:** Comprehensive LC/MS, GC/MS and <sup>1</sup>H NMR urine metabolic fingerprinting in bladder cancer

**Authors:** Julia Jacyna, Renata Bujak, Stéphane Balayssac, Aleksandra Sawicka, Malgorzata Patejko, Marcin Markuszewski, Myriam Malet-Martino, Marcin Matuszewski, Roman Kaliszan, Michal Markuszewski,

**Presenting Author Affiliation:** Medical University of Gdansk

**Abstract Submission:**

Bladder cancer (BCa) constitutes one of the 10 most frequent types of cancer worldwide. Currently applied methods for the detection of BCa require the use of specialist equipment, may cause patients' discomfort and most of all - are adopted when disease symptoms are observed, mostly at the late stage of the disease. Therefore, specific and non-invasive diagnostic method for early diagnosis of BCa is needed. Metabolomics seems to be a great tool in searching for new potential markers of BCa and explanation of its pathomechanisms.

Urine samples obtained from BCa patients (muscle invasive, high grade BCa, n=24) and healthy volunteers (n=24) were analyzed with the use of 3 complementary techniques: RP and HILIC HPLC-TOF/MS in positive and negative ionization modes, GC-QqQ/MS in a scan mode and <sup>1</sup>H NMR. After data treatment (deconvolution, filtration and normalization), the obtained datasets were statistically analyzed using univariate and multivariate statistical analyses to select metabolites which represented statistically significant differences between compared groups. The identification of selected metabolites using publicly available databases allowed to provide a list of putative markers. The selected metabolites (e.g. uric acid, hippuric acid, glutamine, phenylacetylglutamine, pipecolic acid, acetylspermidine, tyrosine, dodecanamide or hydroxytryptophan) can play crucial role in pathomechanisms underlying BCa.

This study shows the potential of metabolomics approach for explanation of BCa mechanisms. Nevertheless, it should be emphasized that the obtained results are preliminary and further validation on larger set of samples is required.

**Acknowledgements**

The project was supported by the National Science Centre granted on the basis of the decisions number DEC- 2012/05/B/NZ7/03293 and 2012/07/E/NZ7/04411.

**Poster #: 134**

**Abstract #: 2585**

**Abstract Title:** Exploration of Tumor Metabolism in Hereditary Kidney Cancer Syndromes Using Stable Isotope-Resolved Metabolomics

**Authors:** Daniel Crooks, Teresa W.-M. Fan, Andrew Lane, W. Marston Linehan,

**Presenting Author Affiliation:** National Institutes of Health

**Abstract Submission:**

Once thought of as a single disease, renal cell carcinoma (RCC) is now known to be several different types of cancer that are characterized by different genetic mutations, histologies, and responses to therapy. Many gene mutations in kidney cancer are known to have a direct and profound effect on cell metabolism, including oxygen sensing by the HIF pathway (VHL), nutrient sensing via the mTOR and other pathways (FLCN, MET, TFE3), and energy sensing as a result of direct disruption of the Krebs cycle (FH, SDH). We are utilizing stable isotope-resolved metabolomics (SIRM) to investigate altered metabolism in patient-derived cultured RCC cells, tumor xenografts in mice, thin tissue slices obtained during surgery, and intraoperative <sup>13</sup>C-glucose infusion in patients to explore the unique metabolic phenotypes associated with the various genetic lesions that cause kidney cancer in humans. Untargeted isotopologue distributions in polar and non-polar metabolites are determined in extracts of cancer and normal kidney using <sup>1</sup>H and <sup>13</sup>C NMR spectroscopy and multiple mass spectrometry modalities to define the metabolic reprogramming in different RCCs. Preliminary analyses in fumarate hydratase (FH)-deficient patient tumors and cells have demonstrated that FH-deficient tumors contain a significant pool of fumarate that is not derived directly from the Krebs cycle via the succinate dehydrogenase reaction. These findings are being used to evaluate the effect of novel therapeutic approaches for kidney cancer that are tailored to the distinctive metabolism and genotype found in the diverse array of genetically-defined kidney cancers.

This work was funded by the National Cancer Institute, Center for Cancer Research and NIH 1R01ES022191-01 and 1U24DK097215-01A1

**Poster #: 135**

**Abstract #: 2014**

**Abstract Title:** Microscopic MALDI-imaging mass spectrometry in intestinal tumors of Apc mutant mice using two-step matrix application

**Authors:** Shuichi Shimma, Satoko Osawa, Yasushi Kojima, Masahiro Aoki, Tomoyoshi Soga,

**Presenting Author Affiliation:**

**Abstract Submission:**

In imaging mass spectrometry (IMS), ionization efficiency and high-spatial resolution are essential to obtain precise distribution information in tumor tissues. Especially in MALDI-IMS, appropriate matrix application is required to achieve these purposes. To improve ionization efficiency, two-step matrix application method for  $\alpha$ -CHCA and direct spraying application method of 2, 5-DHB containing ammonium sulfate (AS) were reported. On the other hand, one drawback of MALDI-IMS in microscopic imaging (less than 20  $\mu\text{m}$ ) is matrix crystal morphology. Large matrix crystals become shade in imaging results. Since matrix crystals via two-step method become submicron scale, we evaluated the two-step application procedure on AS-contained 2,5-DHB as well as  $\alpha$ -CHCA in high-spatial resolution MALDI-IMS.

Tissue sections of intestinal tumor of Apc mutant mice were prepared at 8  $\mu\text{m}$ . 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  $\alpha$ -CHCA prior to matrix spraying using iMLayer (Shimadzu, Kyoto, Japan). After the deposition process, 100- $\mu\text{L}$   $\alpha$ -CHCA solution (8 mg/mL in 50% methanol/0.1% formic acid) or AS-contained 2,5-DHB solution (8 mg/mL containing 250 mM AS in 50% methanol) were provided with an artistic air-brush. MALDI-IMS was performed using product ion detection mode in iMScope (Shimadzu, Kyoto, Japan). In this study, target molecules were S-adenosylmethionine (SAM) and CDP-choline.

Comparing with a histological image of HE-stained serial section, obtained high-spatial resolution ion image (5  $\mu\text{m}$ ) revealed that SAM tended to be accumulated inside the stromal region.

In CDP-choline detection, we successfully obtained high signal-to-noise ratio mass spectra of CDP-choline and high spatial resolution ion distribution images. According to the distribution, CDP-choline provided characteristic distribution in the colon tissue.



**Poster #: 136**

**Abstract #: 2289**

**Abstract Title:** Comparative tissue metabolomic and proteomic analysis of Prostate Cancer subtypes

**Authors:** Ilona Dudka, Elin Thysell, Henrik Antti, Anders Bergh, Pernilla Wikström, Gerhard Gröbner,

**Presenting Author Affiliation:** Department of Chemistry, Umeå University

**Abstract Submission:**

Prostate cancer has very heterogeneous phenotypes ranging from indolent asymptomatic cases to aggressive life threatening and lethal forms. Understanding how cancer subtypes derive energy and necessary building blocks is of fundamental importance for development of appropriate therapies and diagnostic approaches.

We undertook an extensive analysis of human prostate tissue employing combined metabolomics and proteomics analysis to determine significant differences between ERG Rearrangement-positive and -negative prostate cancer. In our workflow we did histologic and comprehensive metabolomics analysis starting from fresh-frozen tumor biopsies from 16 patients obtained at radical prostatectomy. Biopsies were cut into smaller pieces (approx. 2x2 mm, n=129) and metabolites were measured by <sup>1</sup>H HR MAS NMR on the intact tissue. Subsequently, direct analysis of the tissue extract (Brown et al., 2012) was performed by using LC/MS. Extracted tissue was paraffin-embedded, stained by immunohistochemistry and annotated as ERG-pos (n=48) or ERG-neg (n=81). Proteomic analysis had been previously done with LC-MS/MS on the Q-Exactive mass spectrometer in a separate sample cohort (Iglesias-Gato et al., 2015) that was now annotated according to ERG rearrangement (ERG-pos, n=12 ERG-neg, n=15).

We observed significant differences in metabolite pattern between ERG Rearrangement-positive and -negative prostate cancer tissue. We found that levels of several acylcarnitines were increased in ERG Rearrangement-positive prostate cancer. Moreover, most enzymes involved in  $\beta$ -oxidation, i.e. CPT2 were decreased in ERG-positive tissue. This might explain the accumulation of acylcarnitines and also suggest decreased oxidation of the acyl-CoAs in ERG-positive tumors. The ERG Rearrangement-positive group also showed markedly increased levels of metabolites belonging to the purine catabolism. Disturbance of purine metabolism would suggest increased DNA damage and greater oxidative stress in ERG-pos tumors.

Presented combination of metabolomic and proteomic data show markedly different biochemistry of prostate tumors with respect to ERG rearrangement status, which might have profound implications for designing new targets for this disease.

**Poster #: 137**

**Abstract #: 2327**

**Abstract Title:** Three-dimensional MALDI Imaging to Understand Metastasis in Pediatric Medulloblastomas

**Authors:** Facundo Fernandez, Martin Paine, Danning Huang, Jingbo Liu, Shane Ellis, Ron Heeren, Tobey MacDonald,

**Presenting Author Affiliation:** School of Chemistry and Biochemistry. Georgia Inst

**Abstract Submission:**

Medulloblastoma (MB) is the most common and malignant brain tumor in children with a high propensity to metastasize throughout the central nervous system (CNS). The malignant nature of this cancer translates to 30-40% of children relapsing with terminal metastatic CNS disease, even after the use of aggressive radiation therapy. However, those that don't relapse still suffer permanent and debilitating neurocognitive impairment from the treatment. By investigating tumor tissue samples from a sonic hedgehog MB mouse model, biomarkers may be identified that can be used to predict the risk of malignancy, and help direct less radical treatments for patients with lower propensity of CNS metastasis.

Mass spectrometry imaging was employed to investigate sagittal tissue sections of the whole brain and spinal cord from MB mice. Tissue sections (10  $\mu\text{m}$  thick) were collected throughout the volume of the mouse CNS. The sections were coated with norharmane matrix and analyzed in negative-ion mode by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS) on a rapifleX time-of-flight mass spectrometer (Bruker, Germany) equipped with a 10 kHz laser and 50 pixels/second scanning speeds, allowing 3-dimensional imaging data to be acquired on a feasible time scale. Data were acquired between  $m/z$  400 – 1600 with a spatial resolution of 50  $\mu\text{m}$ , creating 2-dimensional MALDI-MS images for each section. These were compiled to form a 3-dimensional MS image using the SCiLS lab software (Bruker, Germany) and analyzed using the unsupervised clustering algorithm probabilistic latent semantic analysis (pLSA).

3-D visualization of metabolites associated with a primary tumor that metastasized compared to a non-metastatic primary tumor enabled identification of lipids and other metabolites uniquely associated with the biological pathways of metastasis. Comparison of imaging data with metabolome alterations detected in mouse serum via high resolution ultra-high performance liquid chromatography-MS metabolomics approaches enabled a more integral view of the alterations associated with MB.

**Poster #: 138**

**Abstract #: 2135**

**Abstract Title:** Metabolomic Changes Associated with Postmenopausal Hormone Therapy

**Authors:** Victoria Stevens, Ying Wang, Brian Carter, Mia Gaudet, Susan Gapstur,

**Presenting Author Affiliation:** American Cancer Society

**Abstract Submission:**

Postmenopausal hormone (PMH) therapy is typically used to ease adverse symptoms that can occur during and after menopause. PMH use also affects risk of a number of other health conditions, including blood clots, stroke, osteoporosis and breast and endometrial cancers. To investigate metabolic changes associated with PMH use, metabolomic profiling was done on plasma samples collected between 1998 and 2001 from 174 postmenopausal women enrolled in the American Cancer Society's Cancer Prevention Study II Nutrition Cohort. PMH users (n= 93) reported exogenous hormone use on the day or day before blood draw whereas nonusers (n=81) reported no hormone use at all. Metabolomic profiling was done by Metabolon Inc. using ultrahigh performance liquid chromatography-tandem mass spectroscopy (UPLC-MS/MS). Levels for 576 known metabolites were scaled to the median and normalized through log-transformation. Logistic regression analyses controlling for age, body mass index, and smoking identified 86 metabolites statistically significantly associated with PMH use after accounting for multiple comparisons (false discovery rate<0.05). The levels of 18 amino acids were lower in PMH users than in non-users, including several intermediates involved in glycine and serine metabolism and in the urea cycle. Fumarate, a urea cycle product that is used in energy production, was also lower in PMH users. Levels of the thyroid hormone thyroxine and the adrenal hormone cortisol were higher in PMH users than in non-users. Carnitine and a number of acylcarnitines were lower in PMH users whereas levels of several glycerophospholipids were higher in this group. Other metabolites that differed by PMH use included sphingolipids, several peptides, and the vitamin retinol. Replication of these results in a separate study population will be done. Metabolomic differences between recent users and non-users provides some insight into how PMH use might give rise to the observed health effects.

**Poster #: 139**

**Abstract #: 2489**

**Abstract Title:** Met-SAMoA: Metabolic Screening of Antimicrobial Mode of Action

**Authors:** Djomangan Ouattara, Andrei BUNESCU, Xavier MENICHE, Emeline BILIAUT, Christelle BOISSE, Jennifer TOMBOSCO, Frederic BEQUET,

**Presenting Author Affiliation:** Researcher

**Abstract Submission:**

Deciphering the mode of action (MoA) of a new antimicrobial compound is a long and expensive process that requires several different technologies. Moreover, characterization of the MoA often happens in the late stages of the drug development process, leading to a delayed selection of drug candidates.

Here we present a metabolomics-based approach to predict the novelty of a drug MoA, using MS fingerprints and NMR footprints of bacterial strains exposed to antimicrobial compounds and based on the statistical comparison of metabolic signatures between the known and the new candidate drugs MoA.

In this context, we developed a platform, Met-SAMoA (Metabolic Screening of Antimicrobial Mode of Action), compatible with SOPs applied in drug development and with biosafety L2 requirements. It combines normalized microbiology experiments for anti-microbial testing in the 96-well format, high throughput automated sample preparation and NMR/MS shotgun analysis for metabolic foot/fingerprints generation. It also includes a bioinformatics workflow consisting in a database containing the metabolomics foot/fingerprints from all tested antimicrobials and predictive multivariate models using statistical machine learning algorithms for the prediction of the MoA of new compounds.

The Met-SAMoA platform is fully operational, adaptable to other pathogens and available for future MoA evaluation of new drugs candidates.

**Poster #: 140**

**Abstract #: 2512**

**Abstract Title:** Multi-omic data integration via constraint-based modeling to unveil metabolic alterations in prostate cancer associated to a chronic exposure to endocrine disruptors

**Authors:** Igor Marín de Mas,

**Presenting Author Affiliation:** CSIC

**Abstract Submission:**

A large number of studies highlight a dose-effect relation between endocrine disruptors (EDs) and tumor progression and malignancy. However our understanding of the effects of chronic exposure to non-lethal concentrations of EDs in cancer metabolism still remains limited.

Here, we study the metabolic profile of DU145 prostate cancer cells before and after a chronic exposure to different EDs (chlorpyrifos (CPF), aroclor 1254 and aldrin), that is reported to induce changes in the lipidic profile and to enhance the malignant phenotype of DU145 cells [Bedia et al., 2015].

To increase our understanding of the metabolic processes underlying these changes, we use constraint-based modeling approaches to integrate the transcriptomic and lipidomic data of DU145 cells in the different conditions into a genome-scale metabolic network reconstruction analysis.

First, we use a number of publicly available databases to enrich and expand the lipid-associated pathways of one of the most recent reconstructions of the human metabolism [Adil Mardinoglu et al. 2014]. This step is aimed to generate a model that enables a better integration and analysis of the lipidomic profile of DU145 cells.

Next, we integrate transcriptomic and lipidomic data into the expanded reconstruction of human metabolism by applying constraint-based modeling methods (CBM). These approaches permit to: i) infer the activity state of the metabolic reactions and ii) define a space of experimentally supported metabolic flux solutions. These CBMs aim to maximize the similarity between the activity state of the metabolic network and gene expression [Shlomi et al. 2008], involving the experimentally measured metabolites [Schmidt BJ et al. 2013], while satisfies the stoichiometric and thermodynamic constraints embed in the model.

Our study will permit to decipher the metabolic mechanisms underlying malignant changes associated to chronic exposure to endocrine disruptors in prostate cancer with potential clinical and environmental applications

**Poster #: 141**

**Abstract #: 2305**

**Abstract Title:** Role of 9-cis Retinoic Acid in Inducing Epithelial to Mesenchymal Transition in Human Pancreatic Ductal Adenocarcinoma Cell Lines

**Authors:** Tatiana Altadill, Prabhjit Kaur, Siddheshwa Chauthe, Talgat Nurkas, Olga Timofeeva, Amrita K Cheema,

**Presenting Author Affiliation:** PhD student

**Abstract Submission:**

Pancreatic ductal adenocarcinoma (PDAC) ranks fourth among all cancer causing deaths in the United States. Poor prognosis can be explained by asymptomatic pathogenesis of PDAC before late stages of cancer advancement and metastasis, scarcity of effective therapeutic approaches and complications of surgical tumor removal due to importance of pancreas as an organ. Enhanced migratory and invasive properties of PDAC cells are conditioned by genetic alterations and TGF- $\beta$  induced Epithelial to Mesenchymal Transition (EMT).

We used high resolution MS based untargeted metabolomics profiling to determine metabolic response to TGF- $\beta$  treatment we found that TGF- $\beta$  induced EMT in PDAC cell lines led to an increase in intracellular Retinoic Acid (RA). However, TGF- $\beta$  induced up-regulation of metabolites and expression of the invasive phenotype was only displayed in the PANC-1 cells harboring mutated K-RAS and not in PANC02.13 that is wild type for K-RAS. In order to validate increase in metabolites after TGF- $\beta$  treatment used a combination of SID-MRM-MS based targeted MS as well as biochemical analysis. We also demonstrate the effects of direct treatment with 9-cis-retinoic acid (9-cis-RA) and all-trans-retinoic acid (ATRA) with respect to EMT regulation. Collectively, these findings indicate that direct treatment with ATRA yielded no significant difference in inducing differentiation TGF- $\beta$  induced EMT is dependent on the KRAS mutation and finally, 9-cis-RA indeed induces differentiation and loss of E-cadherin but may act independently of TGF- $\beta$  via an unconventional signaling pathway. Detailed mechanistic studies to further characterize the role of 9-cis-RA in facilitating EMT signaling will be performed. These results demonstrate the power of metabolomics for studying the molecular basis of cancer progression and metastasis.

**Poster #: 142**

**Abstract #: 2484**

**Abstract Title:** Differential analysis of lung cancer patients' and healthy volunteer's serum samples using ICP-MS

**Authors:** Runhan Yan, Xiaowei Fang, Zhenglong Bi, Huanwen Chen,

**Presenting Author Affiliation:** East China University of Technology

**Abstract Submission:**

Currently, the early diagnosis and treatment of cancer are mainly depended on the sensitive detection of organic molecules and/or bio macromolecules, but few efforts have been made to detect trace metal elements. In fact, trace metal elements serve vital roles in all of the life activities since they are important components of all organisms. Thus it is essential to pay more attention on the relationship between the distribution of elements in organisms and the occurrence and development of cancer disease.

Directly diluted serum samples were directly infused to ICP-MS, generating the mass spectrum recorded in the mass range of  $m/z$  10-300. A total of 400 serum samples (e.g., 200 donated from lung cancer disease, and 200 from healthy volunteers) were analyzed, resulting in a series of mass spectral fingerprints of metal elements in each serum sample. In order to visualize the difference between the two kinds of samples, principal component analysis has been performed based on the data of the metal elements tested.

The experimental data show that differential elements in terms of abundances were found in the two types of serum samples. The signal levels of Fe (56) detected from the healthy serum samples were significantly higher than that of Fe (56) found in the samples of lung cancer patients. On the other hand, the intensity of Cu (63) in the serum samples of the healthy people were dramatically lower than that of Cu (63) in the serum of the lung cancer patients. Further more, the abundance ratio of Fe/Cu was about 1.3 for the serum samples of lung cancer, which was much lower (2 times) than that for healthy people. Similarly, abundance ratios for many other elements including Na, Ca, K etc. have also shown the same trend in correlation with the lung cancer diseases.

**Poster #: 144**

**Abstract #: 2181**

**Abstract Title:** Metabolomic profiling of blood plasma from pre-diabetic and healthy subjects at the Taiwan Biobank

**Authors:** Chung-ke Chang,

**Presenting Author Affiliation:** Taiwan Biobank, Academia Sinica

**Abstract Submission:**

The Taiwan Biobank (TWB) was established to provide a scientific infrastructure for biomedical researchers and to facilitate the development of precision and personalized medicine in Taiwan. The TWB operates the recruitment and monitoring of a general population cohort with no history of cancer (200,000 individuals) and various chronic disease cohorts of public interest (100,000 patients in total), and also generates population-wide omics data for intramural profiling and extramural research. It is hoped that this “big data” approach will eventually enable researchers to develop improved stratagems for disease prevention, make more accurate predictions about disease progression and eventually lead to the evolution of individualized therapy. Here we report our first foray into nuclear magnetic resonance (NMR)-based metabolomics using TWB blood plasma specimens from the general population cohort, with a focus on identifying metabolic differences between pre-diabetic and healthy Taiwanese subjects of Han Chinese descent. We acquired spectra for both whole plasma and plasma filtrate from over 200 specimens and carried out quantitative analysis for 50 metabolites in each specimen. Preliminary analyses of our data suggest that there is little difference amongst the metabolomic profiles of subjects of various ages within each sex, provided that they lead a healthy lifestyle. Interestingly, pre-diabetic females in certain age groups appear to have plasma metabolomic profiles close to those from control subjects, whilst pre-diabetic males from these same age groups generally had more distinct profiles compared to those from control subjects. We discuss these findings in the context of our current knowledge about type 2 diabetes and the potential of metabolomics studies using population cohorts.



**Poster #: 145**

**Abstract #: 2473**

**Abstract Title:**  $^1\text{H}$  NMR-based global metabolomic analysis in diabetic nephropathy mice

**Authors:** Jin Seong Hyeon, Hunjoo Ha, Geum-Sook Hwang,

**Presenting Author Affiliation:** Korea Basic Science Institute

**Abstract Submission:**

Diabetic nephropathy (DN) is one of the most severe microvascular complications in diabetic patients and is the lethal diseases in patients with end-stage renal disease. Plasma and urinary metabolomic profiling in the mouse model of DN was applied to investigate metabolic differences between early DN and advance DN.

$^1\text{H}$ -NMR spectroscopy was used to profile biofluid samples of db/m and db/db mice at the age of 8 and 20 weeks. Multivariate analyses were performed to examine metabolic differences between control and DN groups. The spearman correlation analysis was performed to investigate in correlations of metabolites between serum and urine.

When compared with control group, DN group showed changes in urinary metabolite patterns over time. In addition, levels of the urinary metabolites were significantly changed in the DN group compared with control group. The significantly perturbed metabolites were related to metabolism of carbohydrates and carbohydrate conjugates, TCA cycle intermediates, and amino acid.

In addition metabolic profiles of serum showed difference in metabolic patterns between control and DN groups.

In this study, we investigated the alterations of metabolic signature in the mouse model with early and advance DN. Our study may provide a understanding of metabolic perturbation related to progress of DN.

**Poster #: 146**

**Abstract #: 2179**

**Abstract Title:** Metabolomic analyses of the effects of statins on healthy human cell lines reveal an impaired amino acid metabolism

**Authors:** Janina Tokarz, Gabriele Moeller, Martin Hrabe de Angelis, Jerzy Adamski,

**Presenting Author Affiliation:** Helmholtz Zentrum Muenchen, Institute of Experimen

**Abstract Submission:**

High levels of low density lipoprotein (LDL) and low levels of high density lipoprotein (HDL) promote atherosclerosis and are strongly associated with cardiovascular diseases. Atherosclerosis progression can lead to myocardial infarction, stroke, or other peripheral vascular diseases. To lower the levels of LDL and therewith the risk of cardiovascular diseases, statins have been developed. The competitive inhibitors of the 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) lower the endogenous cholesterol biosynthesis. Statins are mostly safe however, side effects with different severity can occur, like hepatic inflammation, an increased risk for diabetes, muscle inflammation (myositis), and destruction of muscle cells (rhabdomyolysis), which can lead to life-threatening kidney injury. Lethal cases upon cerivastatin therapy caused the withdrawal of the drug in 2001.

Our goals are the understanding of the functional network underlying the statin action, and the identification of unintended molecular targets of statins by the use of metabolomics. Furthermore, we aim to identify the factors which distinguish a safe statin from a risky statin.

We used cerivastatin, simvastatin, pravastatin, and atorvastatin to treat the human cell lines THLE-2 and HK-2, which are derived from healthy liver and kidney, respectively. After determination of the cell line specific dose-response curve based on cell viability, we used the statin in their respective EC50 and EC80 concentrations to treat the cells. We performed targeted metabolomics analyzing amino acid and acylcarnitine levels by using the adapted Newborn Screening Kit (ChromSystems). The metabolomics data was normalized to cell number determined by an in-house fluorescent-based normalization method and subjected to statistical analyses. We found that cells treated with different statins clustered differently in Principal Component Analyses. In Random Forest analyses, branched chain amino acids as well as proline, aspartic and glutamic acid were ranked among the top features. Those metabolites were differentially regulated in a statin- and concentration-dependent manner.

**Poster #: 147**

**Abstract #: 2458**

**Abstract Title:** Metabolic profiling of human heart affected by dilated cardiomyopathy

**Authors:** M Mimmi, Angela Caragnano, Nicoletta Finato, Sandro Sponga, Ugolino Livi, Alessandra Corazza, Gennaro Esposito, Antonio Beltrami,

**Presenting Author Affiliation:** Dept. of Med. Biol. Sciences, University of Udine

**Abstract Submission:**

Idiopathic dilated cardiomyopathy (iDCM) is a primary heart muscle disease characterized by cardiac enlargement and impaired systolic function in the absence of detectable causes. In just 20-30% of cases, a genetic cause can be identified. Moreover, the pathogenetic mechanisms are not completely understood. [Beltrami C.A. et al. (1994). Academic Press]

Previous data suggest that DCM is accompanied by a loss of proteostatic control, that results in the accumulation of amyloid substance. [Gianni D. et al. (2010) Circulation]

Unpublished data from our laboratory, on 50 end-stage iDCM failing hearts, have shown an impairment of the autophagy/lysosomal pathway, coupled with the accumulation of dysfunctional mitochondria, and increased oxidative stress.

Aim of the present study was to verify whether alterations of cardiac metabolism could play a central role in the genesis and evolution of iDCM.

Hydro-soluble metabolites, extracted from biopsies of 12 hearts donated for transplantation and 13 DCM hearts, were analyzed by 1D <sup>1</sup>H-NMR spectra. A statistical analysis was performed on the resulting dataset with the Metabo-Analyst platform. [Xia J. et al. (2015) Nucl. Acids Res.]

Preliminary results from univariate (T-test) and multivariate (PLS-DA) analysis confirm the expected shift from oxidative to glycolytic metabolism within heart failure (increase of pyruvate and lactate) and shows a higher level of branched chain amino acids (BCAA) in patients.

BCAA are involved in different metabolic pathways that resulted perturbed in hypertrophic and dilated cardiomyopathies. Moreover BCAA are among the most powerful controllers of the activity of mTOR, a key factor in the dysregulation of autophagy-lysosomal axis, [Y. Huang, et al. (2011). Cardiovascular Research]. This pathway is likely to be at the center of inflammation and failure in iDCM.

**Poster #: 148**

**Abstract #: 2171**

**Abstract Title:** Urinary metabolomics approaches in metabolic syndrome based liquid chromatography-electrospray ionization/mass spectrometry

**Authors:** Jeongae Lee,

**Presenting Author Affiliation:** Korea Institute of Science and Technology

**Abstract Submission:**

Metabolic syndrome (MetS) is defined by a clustering of biochemical and clinical characteristics, including obesity and insulin resistance. It is associated with an increased risk of both diabetes and cardiovascular diseases. Recently, MetS is a common clinical condition even higher in older age groups with a prevalence variation. In this investigation, a non-targeted UPLC-QTOF/MS and multivariate analysis were applied to human urine from control (N=163, age  $50.92 \pm 15.39$  years) and patient (N=182, age  $60.59 \pm 11.60$  years) using both reversed-phase liquid chromatography (RPC) and hydrophilic interaction chromatography (HILIC). The partial least-squares discriminant analysis (PLS-DA) model was generated from metabolic profiling data and the score plots showed a significant difference in patient with metabolic syndrome. The most differential metabolites were detected from four modes (a total of 240 (VIP > 1) out of 10940 variable ions from RPC positive ionization mode 380 (VIP > 1) out of 10932 variable ions from RPC negative ionization mode 171 (VIP > 1) out of 1510 variable ions from HILIC positive ionization mode 114 (VIP > 3) out of 7172 variable ions from HILIC negative ionization mode). Based on pathway analysis, we found metabolic syndrome were associated with amino acid pathway such as tryptophan (Trp), phenylalanine and tyrosine. To determine whether the Trp metabolism variation in metabolic syndrome, specific metabolites involved in the Trp pathway were quantified absolutely using LC-MS/MS. This study showed that high concentration of kynurenine (Kyn), 3-hydroxy anthranilic acid, xanthurenic acid and quinolinic acid as well as the ratio of Kyn/Trp (KTR) were associated with metabolic syndrome. Our explorative investigation indicated that metabolomics can help to clarify unexplored biochemical pathways in metabolic syndrome.

**Poster #: 149**

**Abstract #: 2110**

**Abstract Title:** Metabolomics Profiling of Adult Retinal Pigment Epithelial (ARPE-19) cells After Adding Vitamin D.

**Authors:** Adel Alghamdi, Ali Tohari, Xinhua.Shu@gcu.ac.uk Shu, David Watson,

**Presenting Author Affiliation:** Strathclyde Institute of Pharmacy & Biomedical Sci

**Abstract Submission:**

Metabolomics Profiling of Adult Retinal Pigment Epithelial (ARPE-19) cells After Adding Vitamin D.

Introduction: Vitamin D plays a significant role in human biological processes in addition to the well-known function of calcium regulation. For instance diabetic patients usually suffer from retinopathy and eyesight problems. Studies in both humans and animals have shown that vitamin D levels and genetic variation have a relation with the development of retinopathy. This study examined metabolic changes in adult retinal pigment epithelial (ARPE-19) cells after adding vitamin D in to cells cultured with normal and high glucose levels. Methods: ARPE-19 cells were grown in medium containing either 1 g/L (normal glucose level (NGL), n=12) or 4.5 g/L (high glucose level (HGL), n=12). Six samples from each group were treated for 24 hours with 50 nM of 1 $\alpha$ ,25-Dihydroxyvitamin D3 (NGLD, n=6 and HGLD, n=6). The treated cells were extracted and analysed by LC/MS. Row data splitting and peak identification was carried out using mzMatch prior to metabolite identification using IDEOM. The output file was transferred to SIMCA-P software for statistical analysis and modelling. Results: Orthogonal partial least squares modelling separated both control and treated groups. There were 77 significantly different metabolites in the HGL/NGL in comparison with 75 significantly different metabolites in HGLD/NGLD. For instance, glutamate metabolism and Krebs cycle were significantly down regulated after adding of 1 $\alpha$ ,25-Dihydroxyvitamin D3 (HGLD/NGLD) the opposite effects were observed when vitamin D was not added (HGL/NGL). Conclusion: This study demonstrated that Vitamin D can affect the metabolomic profile of ARPE-19 cells potentially leading to protection of the retina against diabetic retinopathy particularly with regard to preserving glutathione levels.

**Poster #: 150**

**Abstract #: 2186**

**Abstract Title:** Impact of metformin and glucose on the hepatocellular metabolism

**Authors:** Caroline Muschet, Cornelia Prehn, Anna Artati, Gabriele Möller, Martin Hrabe de Angelis, Jerzy Adamski,

**Presenting Author Affiliation:** Helmholtz Zentrum München

**Abstract Submission:**

Within the last decades, type 2 diabetes has become one of the major challenges of modern health care. The readjustment of glucose homeostasis is one of the driving forces in the treatment of this disease. However, molecular mechanisms of interaction between sugar associated pathways and anti-hyperglycaemic drugs are not yet fully understood. More knowledge would be helpful in optimizing diabetes treatment. We therefore addressed the impact of high glucose levels and the interaction of glucose and the (widely prescribed) anti-hyperglycaemic drug metformin.

We were especially interested in the regulation of the hepatocellular metabolism and chose the model cell lines THLE-2 (derived from normal human liver) and Hep G2 (derived from a hepatocellular carcinoma). These cells were grown at physiological (6 mM) or elevated (11 mM) glucose concentrations either in the absence or in the presence of metformin. Cells were subsequently analyzed by a metabolomics approach.

For Hep G2 cells, elevating the glucose levels correlated with significantly increased cell numbers, and metformin treatment led to a significant decrease in cell number, regardless of glucose concentrations in the culture medium. Although, the same trends were observed for THLE-2 cells, they were found to be less pronounced than in Hep G2. In addition, both, the glucose concentration as well as the metformin treatment correlated with vast alterations in the hepatocellular metabolomes. The effects were seen in both model cell lines however, more pronounced in Hep G2. Taking a closer look at the effected metabolites, changes in the hexose and amino acid levels as well as alterations in the metabolization (e.g. desaturation and hydroxylation status) of lipids were observed.

**Poster #: 151**

**Abstract #: 2200**

**Abstract Title:** Plasma metabolites profile in Inuit adults from Nunavik (northern Quebec) with metabolic syndrome

**Authors:** Cynthia Roy, Pierre-Yves Tremblay, Elhadji Anassour-Laouan-Sidi, Michel Lucas, Pierre Ayotte,

**Presenting Author Affiliation:** Centre Hospitalier Universitaire Research Centre

**Abstract Submission:**

The metabolic syndrome (MetS) is associated with increased risk of cardiometabolic diseases such as type 2 diabetes (T2D). Like other Indigenous populations, the Nunavik Inuit population undergoes dietary and lifestyle transitions that could negatively impact cardiometabolic health in the future. Amino acids (AA) and acylcarnitines (AC) are among the most promising biomarkers for the prediction of T2D. Plasma AA and AC concentrations were shown to be elevated up to 12 years prior to the onset of T2D. Our study aims to assess the hypothesis that MetS can be discriminated by metabolic biomarkers.

Biobanked plasma samples from 914 Inuit adults who participated to the 2004 Inuit Health Survey were analyzed by using a targeted metabolomic method for eight AA and eight AC using UHPLC-Q-TOF-MS and plasma concentrations were compared between MetS individuals and healthy controls using logistic regression analysis adjusted for age, sex and smoking status.

Concentrations of several AA and AC were significantly associated with elevated triglycerides, waist circumference and decreased high density lipoproteins (11, 7 and 9 metabolites respectively adjusted p-values ranging from <0.0001 to 0.0462). The metabolites most strongly associated with these MetS components were the branched-chain and aromatic AA, glutamic acid and methionine as well as acetyl-, propionyl-, butyryl- and isobutyrylcarnitine. In contrast, plasma AA and AC were not significantly associated with hypertension or fasting glycemia.

In this cross-sectional study, we observed that plasma concentrations of several AA and AC are linked to adiposity and plasma lipid dysregulation in Inuit adults. The capacity of these metabolic biomarkers to predict the future risk of cardiometabolic diseases in this population will be addressed through a follow-up study in 2017.

**Poster #: 152****Abstract #: 2216**

**Abstract Title:** Association of age and sex with plasma and urine metabolite profiles from healthy humans

**Authors:** Manuela Rist, Alexander Roth, Lara Frommherz, Christoph Weinert, Diana Bunzel, Carina Mack, Björn Egert, Achim Bub, Ralf Krüger, Benedikt Merz, Sabine Kulling, Bernhard Watzl,

**Presenting Author Affiliation:**

**Abstract Submission:**

It has been shown that the human metabolome is influenced by age and sex. Most studies, however, have based their findings on either plasma or urine samples and have used only one analytical technique. Therefore, the goal of this study was to identify metabolite patterns that are associated with age or sex of healthy humans based on plasma and urine samples that were analysed with a combination of different analytical techniques.

In the cross-sectional KarMeN study (Karlsruhe Metabolomics and Nutrition) 301 healthy male and female participants, aged 18 – 80 years, were included. Volunteers were examined under strictly standardized conditions. In addition to the determination of numerous anthropometric and functional parameters, fasted plasma and 24h urine samples were collected and analysed by targeted and untargeted metabolomics methods using GC×GC-MS, GC-MS, LC-MS and NMR. Predictive modelling was applied on the combined data using the following machine learning algorithms: SVM, glmnet and PLS.

Based on combined metabolite profiles, it was possible to predict age in men and women with high accuracy from urine as well as plasma. Besides a number of unknown analytes, some metabolites important for this prediction could be identified, such as creatinine and sedoheptulose in urine. Classification of volunteers according to sex was also possible with high accuracy based on urine and plasma metabolite profiles. Plasma metabolites important for correct classification included creatinine and the branched-chain amino acids valine, leucine and isoleucine.

These results confirm that age and sex are associated with metabolite patterns of healthy humans. Many of the metabolites identified in the present study have been described in the context of age and sex before, pointing at robust associations with age and sex. These need to be considered in nutritional metabolomics studies that include volunteers of both sexes and different age ranges.



**Poster #: 153**

**Abstract #: 2452**

**Abstract Title:** Diabetes during Pregnancy Produces Placental Metabolic Alterations at Term

**Authors:** Jacquelyn Walejko,

**Presenting Author Affiliation:** University of Florida

**Abstract Submission:**

Diabetes is a major cause of maternal and neonatal morbidity and mortality during pregnancy. Metabolic changes occur in healthy women throughout pregnancy. Yet, there is little evidence on how altered metabolism, due to pre-existing or gestational diabetes, affects the placenta and fetus. The objective of this study was to determine metabolic alterations in the term placenta in women with diabetes and to detect whether these metabolic alterations can be observed in maternal urine prior to birth.

Women were recruited on admission to the Labor & Delivery Unit at UF Shands Hospital (UF IRB201500007). Urine was obtained at labor onset and within 3 days post-partum. Placental specimens were collected following cesarean delivery (within 20 minutes) from fetal and maternal tissues of the placenta. <sup>1</sup>H-NMR was used to obtain metabolic profiles of urine and placental specimens. Multivariate and univariate statistics were used to determine metabolites that differ between groups.

Principal component analysis revealed increases in citrate and ketones ( $p < 0.05$ ) in diabetic urine ( $n=5$ ) at labor onset compared to control ( $n=7$ ), suggesting alterations in glycolysis and TCA cycle flux. Placental tissue revealed elevations in acetate ( $p < 0.05$ ) in diabetic placentas ( $n=3$ ) adding to evidence of altered glycolysis. Taurine was increased ( $p < 0.05$ ) in control placentas ( $n=2$ ), an amino acid that is essential for preventing apoptosis. Finally, glucose, choline, and glutamine were increased within fetal placental tissue ( $p < 0.05$ ), supporting evidence that the placenta aids in producing these metabolites for the fetus.

Preliminary evidence presented here suggests altered metabolism in placentas from diabetic women at term. In addition, these metabolic changes can be detected non-invasively in the urine at labor onset. This study is currently being repeated with a larger population to determine maternal metabolic profiles that are indicative of fetal and placental health with the ultimate goal of improved management of pregnancy in diabetic women.

**Poster #: 154**

**Abstract #: 2064**

**Abstract Title:** Novel insights from a metabolomics study of heart failure in a mouse model

**Authors:** Oliver Müller, Markus Heckmann, Thomas Gerken, Henning Witt, Sandra González Maldonado, Andreas Jungmann, Julia Kreusser, Johannes Backs, Philipp Schatz, Hugo Katus, Norbert Frey,

**Presenting Author Affiliation:** Universitätsklinikum Heidelberg

**Abstract Submission:**

Heart failure is a complex disorder that affects increasing numbers of patients and poses an extreme burden on public health systems. Unfortunately, the underlying mechanisms are poorly understood, delaying the development of early diagnostic screens and effective therapies. We therefore set out to study alterations of gene expression and metabolic changes associated with early stages of the disease in a surgical mouse model of heart failure.

Mice were subjected to transverse aortic constriction or a sham procedure and sacrificed either two, four or six weeks after the procedure. Samples of heart tissue were collected and analysed using mRNA microarrays as well as metabolite profiling.

Previous reports of impaired mitochondrial beta-oxidation and a “metabolic switch” towards sugar oxidation in heart failure were confirmed by the reduced expression levels of metabolic enzymes in our data set. Various enzyme isoforms untypical of healthy heart tissue were expressed in failing hearts. Failing hearts also produced metabolites uncharacteristic of healthy hearts.

Even early stages of heart failure induced by transverse aortic constriction present with significant changes in metabolite levels as well as altered expression levels and identities of mRNAs expressed in heart tissue. The data gathered in this study contain valuable information on processes occurring early in the failing heart to better understand the associated pathogenic mechanisms and to guide future developments in diagnostic and therapeutic procedures.

**Poster #: 155**

**Abstract #: 2164**

**Abstract Title:** Metabolomic signatures of bean-to-rice ratio in the Costa Rica Study

**Authors:** Stella Aslibekyan, Anh Do, Alla Karnovsky, Maureen Kachman, Bill Duren, Alexander Raskind, Tanu Soni, Charles Burant, Ana Baylin, Hannia Campos,

**Presenting Author Affiliation:** University of Alabama at Birmingham

**Abstract Submission:**

The ratio of beans to white rice in the diet is a robust predictor of metabolic syndrome in Hispanic populations. To gain insights into the underlying physiologic mechanisms, we conducted untargeted metabolic profiling and lipidomic profiling of plasma samples from 80 healthy Costa Rican adults with available dietary information. After quality control procedures, 1302 metabolites and 408 lipids were available for analysis. We log-transformed lipids and metabolites as necessary to achieve normality and tested the associations between dietary beans-to-rice ratio and each plasma variable using linear regression, adjusting for age, sex, and waist circumference. In the metabolomics analysis, phosphatidic acid emerged as the top correlate ( $P$ -value =  $5.1 \times 10^{-5}$ ) of the beans-to-rice ratio. The top lipidomic signals were produced by triacylglycerols, free fatty acids, phosphocholines, and phosphoethanolamines (all associations were negative, with  $P$ -values  $< 0.006$ ). We subsequently tested associations between the features associated with the bean-to-rice ratio and known markers of chronic disease, including plasma lipids and inflammatory markers. The top correlate of the bean-to-rice ratio, phosphatidic acid, was nominally negatively associated with plasma low-density lipoprotein (LDL) cholesterol ( $P = 0.03$ ) but not any other disease markers. Among lipidomic signals, several triacylglycerols, including TG46:1, TG48:1, TG48:2, TG50:2, and TG51:1 were robustly ( $P$ -values  $< 7.0 \times 10^{-5}$ ) positively associated with plasma triglycerides. Sphingomyelins SM35:2 and SM36:1 were positively associated ( $P$ -values  $< 0.004$ ) with plasma LDL and total cholesterol. Phosphatidylcholines PC32:0, PC32:1, and PC34:1 were associated with plasma high-density lipoprotein (HDL) cholesterol ( $P$ -values  $< 0.01$ ), while PC35:3 was associated with LDL and total cholesterol as well as vascular cellular adhesion molecule-1 ( $P$ -values  $< 0.008$ ). In summary, we identified several biologically plausible features associated with a higher ratio of beans-to-rice and metabolic dysfunction markers, suggesting that future metabolomic and lipidomic studies could provide relevant information on biological mechanisms whereby dietary patterns relate to chronic disease.

**Poster #: 156**

**Abstract #: 2217**

**Abstract Title:** UPLC-MS targeted profiling of aromatic phenolic compounds, related with cardiometabolic diseases

**Authors:** Antonis Myridakis, Leanne Nye, Ian Wilson, Marc-Emmanuel Dumas,

**Presenting Author Affiliation:** Imperial College London

**Abstract Submission:**

Gut bacteria (i.e. the gut microbiome, >10 million genes) can substantially affect physiology. However, the bacterial signaling molecules, their host targets and mechanisms are still unclear. Several gut microbial metabolite families have been associated with cardiometabolic diseases in previous metabolomic studies in the mouse and in humans. For example, the gut microbiota are involved in aromatic amino-acid metabolism, degrading tryptophan into a series of indoles (e.g. sulphate, acetate, propionate, lactate and pyruvate) with signaling properties.

The key goals of this study are to develop an analytical method enabling the quantitative determination of a very important gut microbial metabolite family, aromatic phenolic compounds (indoles, cresols etc.) in human urine and plasma samples.

There are two serious challenges to be overcome:

I) the diversity of phenols' physicochemical properties has a number of consequences: the less polar ones are ionized poorly with electrospray ionization (ESI) which results in low sensitivity and the polar ones are not retained effectively by reversed-phase UPLC columns,

II) there is also a requirement to develop an effective clean-up protocol which will reduce substantially matrix effects and column overloading, will prevent the oxidation of phenolic compounds and will be simple and fast, thus appropriate for the analysis of large number of samples

Protein precipitation has been optimized for the sample clean up. Reversed-phase UPLC has been employed for the chromatographic separation of the analytes of interest and tandem mass spectrometry (triple quadrupole) for their detection. Two ionization sources have been compared, ESI and electrospray chemical ionization (ESCI) which offers increased sensitivity for the less-polar compounds. Furthermore, derivatization with dansyl chloride has been evaluated as an alternative solution, which offers both improved retention and sensitivity.

**Poster #: 157**

**Abstract #: 2256**

**Abstract Title:** NEW INSIGHT ON OBESITY AND ADIPOSE-DERIVED STEM CELLS BY COMPREHENSIVE METABOLOMICS

**Authors:** Annalura Mastrangelo, María Panadero, Laura Martín Pérez, Beatriz González Gálvez, Antonia Garcia, Coral Barbas, Francisco Rupérez,

**Presenting Author Affiliation:** Universidad CEU-San Pablo

**Abstract Submission:**

Obesity affects the functional capability of adipose-derived stem cells (ASCs) and their effective use in regenerative medicine through mechanisms still poorly understood. Here we employed a multiplatform (LC/MS, CE/MS, GC/MS) metabolomics untargeted approach to investigate the metabolic alteration underlying the inequalities observed in obese-derived ASCs. The metabolic fingerprint (metabolites within the cells) and footprint (metabolites secreted in the culture medium) from humans or mice, obese and non-obese derived ASCs, were characterized by providing valuable information. Metabolites associated to glycolysis, TCA, pentose phosphate pathway and polyol pathway were increased in the footprint of obese-derived human ASCs by indicating alterations in the carbohydrate metabolism whereas from the murine model, deep differences in lipid and amino acid catabolism were highlighted. Therefore, new insights on the ASCs metabolome were provided that enhance our understanding of the processes underlying the ASCs stemness capacity and its relationship with obesity, in different cell models.

**Poster #: 158**

**Abstract #: 2268**

**Abstract Title:** Non-targeted metabolomics in early vascular ageing syndrome

**Authors:** Michal Markuszewski,

**Presenting Author Affiliation:** Medical University of Gdansk

**Abstract Submission:**

Early vascular ageing (EVA) constitutes premature pathological change in the vascular tree and arterial function [1]. This condition may increase cardiovascular risk and is related to numerous cognitive dysfunctions. The physiopathology of EVA is still not fully explained. In this pilot study, non-targeted metabolomics approach was applied to compare plasma metabolic fingerprints of non-EVA (n=60) and EVA patients (n=60). Analytical measurements were performed with the use of liquid chromatography coupled with time-of-flight mass spectrometry (LC-TOF/MS) in both positive and negative ionization modes. Principal component analysis (PCA) was used in order to check the quality of analyses, assess general trends in the data and detect potential outliers. To select statistically relevant metabolites between non-EVA and EVA patients, a technique called Least Absolute Shrinkage and Selection Operator (LASSO) was applied. As a result, 4 down-regulated metabolites were identified as those contributing the most into group classification. Among them, phosphatidic acid and lysophosphatidylcholine represent signalling lipid mediators involved in various biological processes such as cell proliferation, cell migration, endothelial dysfunction, inflammation, signal transduction and oxidative stress. In EVA group, we observed weak positive however significant correlation between phosphatidic acid and systolic blood pressure ( $r = 0.31$ ,  $p = 0.015$ ), which is known risk factor of intima-media thickening. We therefore conclude that metabolomics may provide new insights into molecular mechanisms and biochemical phenotype of early vascular ageing syndrome.

**Acknowledgements**

The project was supported by the National Science Centre granted on the basis of the decisions number DEC- 2012/07/E/NZ7/04411.

**References**

[1] Nilsson PM. EMJ Diabet. 2015, 3, 80-86.

**Poster #: 159**

**Abstract #: 2339**

**Abstract Title:** A CE-MS based metabolomics study reveals the therapeutic mechanism of Shaofu Zhuyu decoction in a diet-induced obesity mouse model

**Authors:** Jeeyoun Jung,

**Presenting Author Affiliation:** KIOM

**Abstract Submission:**

Metabolic syndrome is a cluster of conditions that increases the risk for the development of cardiovascular disease and type 2 diabetes mellitus. Thus, it is closely related to blood stasis syndrome in Traditional Korean Medicine, and doctors of Korean Medicine utilize a BSS-treated remedy to cure these conditions. In this study, we applied a CE-MS metabolomics approach to investigate the therapeutic mechanism of Shaofu Zhuyu decoction (SFZYD), one of the more famous BSS-treatment remedies, in metabolic syndrome.

Metabolic profiles of sera from a normal chow diet (NC) group, a diet-induced obesity (DIO) group, and a SFZYD-treated DIO group were investigated using CE-MS spectroscopy coupled with multivariate statistical analysis.

The SFZYD treated DIO group showed significantly lower levels of triglycerides (TG) in sera and liver compared with the DIO group. Histopathologic evaluation of liver and adipose tissue demonstrated that SFZYD reduced lipid accumulation in the liver and crown like structures (CLS) in adipose tissue. In addition, MCP-1, IL-6, and PAI-1 in DIO mice were significantly decreased after SFZYD administration. Moreover, principal component analysis (PCA) of metabolic profiling from mice sera revealed a metabolic shift in DIO mice after SFZYD administration toward the NC group. The resultant metabolic profiles demonstrate that SFZYD corrected high fat induced metabolic disturbances, such as energy metabolism and the pentose phosphate pathway, which was related to macrophage recruitment and nucleotide biosynthesis in the DIO group. Additionally, the SFZYD-treated DIO group also showed significant alterations in the levels of tryptophan and tyrosine, which are substrates for neurochemical mediators, such as serotonin and catecholamines. Furthermore, we confirmed that the effects of SFZYD on metabolic regulation and inflammation were related to changes in adipokines, such as adiponectin, and the ratio of adiponectin to leptin.

These data demonstrate that a metabolomics approach may be useful to explore the

**Poster #: 160****Abstract #: 2405****Abstract Title:** Diversity of plasma bile acid profiles in response to dietary challenges**Authors:** Jarlei Fiamoncini, Kurt Gedrich, Tim Broek, Andrianos Yiorkas, Gary Frost, Ben Ommen, Alexandra Blakemore,**Presenting Author Affiliation:** ZIEL – Institute for Food and Health. Technical Un**Abstract Submission:**

**Introduction and Objective** – Bile acids (BA) have recently received considerable scientific interest by their metabolic activity and as ligands of FXR and TGR5. These properties add an important function to their well-known role in lipid digestion and cholesterol homeostasis. In the framework of the Nutritech project, we profiled BA in plasma samples collected from healthy subjects during different dietary challenges.

**Methods** – 72 healthy volunteers underwent an oral glucose tolerance test (OGTT) and a mixed meal tolerance test (MMTT). These challenges were repeated after 13 weeks when 40 volunteers underwent a dietary intervention with 20 % less calories than normal while 32 participants maintained their habitual diet without energy restriction. Most abundant BA were quantified using LC-MS/MS.

**Results** – A large inter-individual variation in plasma BA concentration was observed with fasting BA concentrations varying up to 7-fold amongst individuals. Both the OGTT and the MMTT induced increases in plasma BA concentrations (on average 2-fold in the OGTT and 4-fold during the MMTT), with highly variable changes amongst the subjects. In some cases, BA concentrations were maximal after 1 hour decreasing thereafter, while in other volunteers, maximal concentration of plasma BA were reached only after 6 hours. A third group of volunteers showed maximal BA concentration in the first hour with sustained concentrations of up to 6 hours and thereafter decreasing. In the post-prandial state, clear gender-related differences are also observed as well as an association between plasma BA and GLP-1 concentrations. In order to assess the origins of the variability in BA concentrations in plasma, these results will be analysed in combination with gut microbiota composition and genetic variation in 56 genes encoding proteins involved in BA synthesis and transport.

**Conclusion** – Like no other category of metabolites BA show huge inter-individual variability in plasma levels that remains to be explained.



**Poster #: 161****Abstract #: 2450****Abstract Title:** Circulating 1H NMR based metabolomic profiling associated to proteinuria in diabetic patients**Authors:** Clara Barrios, Jonas Zierer, Sol Otero, Eva Rodríguez, María José Soler, Anna Buxeda, Gabi Kastenmüller, Tim Spector, Cristina Menni, Julio Pascual,**Presenting Author Affiliation:** Hospital del Mar Department of Nephrology, Hospita**Abstract Submission:**

## Background

Diabetic kidney disease (DKD) is the leading cause of chronic kidney disease. Proteinuria, the hallmark of early DKD is a cardiovascular risk factor and the main cause for renal function decline. However its association with blood biomarkers is not well established. The aim of this study is to look for associations between circulating metabolites levels and proteinuria in a diabetic cohort. This might provide more sensitive and specific markers and might help to understand the physiopathological pathways related to renal damage and diabetes.

## Methods:

Absolute concentration of 148 serum metabolites were analysed by 1H-NMR in 445 type2 diabetic subjects with glomerular filtration rate (eGFR) higher than 40mL/min/1.73m<sup>2</sup> and different grade of proteinuria from the Genodiab-Mar cohort. We used linear models adjusting for age, sex, BMI, eGFR, diabetes duration and multiple testing using Bonferroni correction ( $p < 3.4 \times 10^{-4}$ ).

## Results:

We find nine metabolites associated with proteinuria after adjusting for multiple testing. Among those, four triglycerides lipoprotein subclasses positively and four HDL lipoprotein subclasses inversely associated with proteinuria. This supports the idea of a selective loss of small HDL in patients with proteinuria. Interestingly, none of the classic lipid related metabolites were significantly associated with proteinuria in our cohort.

Moreover, we found two amino-acids, which were previously reported to be associated with eGFR, positively correlated with proteinuria, independently of renal function: Glycine ( $\beta$  [95% CI] 2.35 [1.33:3.36]) and Phenylalanine ( $\beta=1.86$  [0.69:3.02]  $p=1.9 \times 10^{-3}$ ). Finally, we identified 3-Hydroxybutyrate, a ketone body metabolite, ( $\beta=0.66$  [0.18:1.14]) ( $p=7.2 \times 10^{-3}$ ) and Apolipoprotein-A, a protective cardiovascular marker, ( $\beta=-2.21$  [-3.63:-0.79]  $p=2.3 \times 10^{-3}$ ) also nominally associated with proteinuria but not with eGFR.

## Conclusion:

This is the largest hypothesis-free approach 1H-NMR based study to investigate the relationship between blood metabolites and proteinuria in type2 diabetes. We find that NMR-based metabolomics

provides insights into the underlying mechanism in the pathogenesis of DKD at metabolic level.

**Poster #: 162**

**Abstract #: 2509**

**Abstract Title:** Curcumin derivative induces energy metabolism and increases intracellular lactate production through AMPK signaling pathway in muscle cells

**Authors:** Nami Kim,

**Presenting Author Affiliation:** Korea Basic Science Institute

**Abstract Submission:**

Curcumin and its derivatives exerts multiple biological and medicinal effects on human health. However, the underlying mechanism actions of curcumin derivative are not completely understood. To identification of metabolic effect of curcumin derivative, <sup>1</sup>H-NMR based metabolome analysis was performed on skeletal muscle C2C12 myoblasts. Principal components analysis (PCA) showed significant separation between absence and presence of curcumin derivative. Curcumin derivative induced phosphorylation of AMP-activated protein kinase (AMPK), which is a key energy sensor and regulates energy metabolism to cellular homeostasis. In addition, curcumin derivative activated p38 mitogen-activated protein kinase (p38 MAPK), this effect blocked by pretreatment with compound C is an AMPK inhibitor. Furthermore, curcumin derivative increased levels of intracellular lactate. Lactate as an important metabolic intermediate, controls expression of several proteins involved in mitochondrial activity and biogenesis. Curcumin derivative-induced lactate stimulated mitochondrial uncoupling protein 1 (UCP1) which acts in expenditure regulation of energy, thermogenesis and fat-browning. Taken together, these results suggest that curcumin derivative presented positive metabolic effects by activating AMPK-p38 signaling pathway and UCP1 by lactate in skeletal muscle cells.

**Poster #: 163**

**Abstract #: 2536**

**Abstract Title:** Coupling Urinary Trihalomethanes and Metabolic Profiles of Type II Diabetes Mellitus: a Case-Control Study

**Authors:** Xanthi Andrianou, Pantelis Charisiadis, Konstantinos Makris,

**Presenting Author Affiliation:** Water and Health Laboratory, Cyprus International

**Abstract Submission:**

Exposures to disinfection by-products, more specifically to trihalomethanes (THM), have been long investigated in population studies. The incorporation of metabolomics in characterizing THM exposures provides a new tool in exposure science, linking external measures of exposures (i.e. from environmental samples) with internal targeted (i.e. urinary levels of THM) and untargeted measurements (i.e. metabolic profiles). A pilot case-control study with diabetic and non-diabetic individuals was conducted to: (i) identify the optimal deconvolution settings for the processing of metabolic fingerprints, and (ii) evaluate the association between differentially expressed metabolites and urinary THM adjusting for the disease status.

First morning urine samples of 23 diabetics and 51 healthy individuals were analyzed. An Agilent 7890A/7000 GC/MS Triple Quad system was used to generate the urinary metabolomics data while THM concentrations had been previously measured. A quality control mixture of fatty acid metabolites was used as markers of the analytical process performance throughout the process. Raw chromatograms were pre-processed in AMDIS using eight different settings combinations for deconvolution and compounds were identified against the Fiehn library (level 2 according to the Metabolomics Standards Initiative). Peak tables were generated using R and processed in MetaboAnalyst. Differentially expressed compounds between cases and controls were used in linear regression analysis as predictors of urinary THM levels adjusting for participant characteristics (i.e. age, body mass index, diabetes status).

Differences in the deconvolution settings led to different number of compounds identified in the same samples. The use of additional metabolites as predictors of urinary THM levels led to improved fit of linear regression models. The metabolites that were regressed onto the urinary THM levels were examined along the biological pathway of THM metabolism in the liver. However, the small sample size of the present study did not favor the generalizability of the results.

**Poster #: 164**

**Abstract #: 2089**

**Abstract Title:** Lipidomic parameters associated with obesity

**Authors:** Christian Klose, Michal Surma, Celine Fernandez, Olle Melander, Kai Simons,

**Presenting Author Affiliation:** Lipotype

**Abstract Submission:**

Obesity is an obvious manifestation of an unhealthy lifestyle that might eventually result in severe conditions such as diabetes type 2 and cardiovascular disease. Therefore, methods for efficient health monitoring are desirable to enable early preventive actions. In order to identify lipidomic markers for obesity, blood serum samples of 571 subjects (mean age = 74 years mean BMI = 28 kg/m<sup>2</sup> 53% female) from the reexamination of the Malmö Diet and Cancer Cardiovascular Cohort (MDC-CC) with sampling performed between 2007 and 2012, were analysed by means of a quantitative high throughput mass spectrometry-based shotgun lipidomics platform. Lipids were extracted from 2 µl of blood serum by a fully automated extraction procedure. Mass spectra were acquired with high-resolution mass spectrometers and lipids identified and normalized with in-house software solutions. Technical variation across the entire sample set was assessed by the inclusion of reference samples and was as low as 9% (c.v.). Out of a total of 1151 lipidomic features, regression analysis revealed about 200 lipidomic features positively or negatively associated with BMI. For example, significant positive associations were found mainly for triglycerides while ether phospholipids were negatively associated with BMI. Furthermore, differential associations within lipid classes could be identified: triglycerides containing long and unsaturated fatty acid moieties were negatively associated with BMI. These results corroborate and extend findings of previous studies. In conclusion, by exploiting a robust and quantitative shotgun lipidomics technology, a multitude of parameters could be extracted from the human serum lipidome that are associated with BMI. These findings open up new opportunities for efficient lipidomic screening.

**Poster #: 165**

**Abstract #: 2189**

**Abstract Title:** Sex Differences in the Association of Phospholipids and Sphingolipids with the Metabolic Syndrome in young adults

**Authors:** Sebastian Rauschert, Olaf Uhl, Berthold Koletzko, Trevor Mori, Lawrence Beilin, Wendy Oddy, Christian Hellmuth,

**Presenting Author Affiliation:** LMU Munich

**Abstract Submission:**

There are differences in the prevalence and severity of diseases between males, females not taking hormonal contraceptives (nhfemales) and females taking hormonal contraceptives (hfemales). The aim of this study was to analyse differences in general and in the metabolic syndrome (MetS) specific metabolic alteration between males, nhfemales and hfemales, to highlight the need to account for disease specific sex differences in metabolomics and interventional studies. The subjects analysed are from the 20 yrs follow-up from the Western Australian Pregnancy Cohort (Raine) Study. 215 plasma metabolites were analysed in 1021 fasted plasma samples by a targeted LC-MS/MS metabolomics approach. Principal Components Analysis between the three groups of males (n=550), nhfemales (n=199) and hfemales (n=272) showed a general discriminating trend between males and hfemales. Regression analysis with a sex\*metabolite concentration interaction was performed for the five indicators of the MetS: waist circumference, HDL-C concentration, triglyceride concentration, glucose concentration and systolic blood pressure as outcome to select the significant metabolites of the interaction. Those selected metabolites were used as predictors in a sex-group stratified analysis to compare the different beta-coefficients and therefore the sex-group dependent associations.

All in all, 127 metabolites were significantly different between males and nhfemales, whereas 95 differed between nhfemales and hfemales. Males and nhfemales mainly differed in SM, LPC, Carn and AA species, whilst nhfemales and hfemales mainly differed in PC, LPC and Carn concentrations. In association with the MetS factors, 43 metabolites have been significantly different in the stratified analysis. The metabolites mainly found different were long chain polyunsaturated species of SMs, PCs and LPCs. Thus, we showed the general and in particular the disease specific sex differences and differences between nhfemales and hfemales in metabolite concentrations. The results highlight the need for sex specific analysis in metabolomics that also takes nhfemales into account.

**Poster #: 166**

**Abstract #: 2260**

**Abstract Title:** Metabolomic analysis on the three stages of diabetes acquisition

**Authors:** Yeseung Lee, Carl Angelo Medriano, Seri Hong, Sun Ha Jee, Youngja Park,

**Presenting Author Affiliation:** Korea University, College of Pharmacy

**Abstract Submission:**

This research aims to apply high resolution metabolomics (HRM) to the detection of early biomarkers relating to the progression of prediabetes. It focuses on determining the metabolomics profiles associated with the progression to and diagnosis of prediabetes in a retrospective study, which can be used to improve the prevention of type 2 diabetes.

The subjects were chosen from a cohort study (KCPS-II) involving 159,844 Korean volunteers. The samples enrolled in the study were classified into three diagnostic groups based on their fasting blood sugar levels: no diabetes mellitus (NDM)(n=15, FBS=84.82±7.61mg/dL), prediabetes (PDM)(n=17, FBS=111.82±7.36mg/dL), and type2 diabetes mellitus (DM)(n=14, FBS=180.43±45.80mg/dL). Serum samples were collected twice for two years. The samples were analyzed using negative mode liquid chromatography-mass spectrometry-based HRM. A multivariate statistical analysis was performed to obtain significantly expressed metabolites using a false discovery rate (FDR) multiple testing correction threshold of  $q=0.05$ .

The HRM screening with bioinformatics identified metabolic differences in all 3 different stages. Findings confirm the increasing glucose level ( $m/z$ : 215.13, [M+Cl]<sup>-</sup>) from NDM to PDM to DM. Even with this result, other compounds found to be significantly affected like Leucine ( $m/z$ : 130.09, [M-H]<sup>-</sup>), Cytosine ( $m/z$ : 110.04, [M-H]<sup>-</sup>) and L-Glutamate ( $m/z$ : 184.00, [M+K-2H]<sup>-</sup>) showed a profile where NDM and DM were of similar levels while PDM levels are either elevated or decreased compared to the other 2 groups. This suggests the importance of PDM stage. The findings suggests that the PDM stage may serve as a critical stage wherein subjects are in a reversible condition which may either be treated back to normal or may proceed to be in an irreversible condition, DM.

**Poster #: 167**

**Abstract #: 2497**

**Abstract Title:** Metabolomics for new colchicine treatment of experimental atherosclerosis

**Authors:** Mario Izidoro, Jesús Mateo de Castro, Jean Paul Vilchez, Alberto Cecconi, Ángeles López-González, Fernanda Rey-Stolle, Jesús Cabello, Coral Barbas, Borja Ibañez, Francisco Rupérez,

**Presenting Author Affiliation:** Centre of Metabolomics and Bioanalysis, Universida

**Abstract Submission:**

Vulnerable atherosclerotic plaque, with respect to stable one, shows bigger lipid core, more macrophages and finer and more marked neovascularization fibrous layer. There was previous evidence that treatment with colchicine, an anti-inflammatory, could revert these changes, increasing plaque stability. Balloon catheter denudation in New Zealand white rabbits fed high cholesterol diet is an extensively validated atherosclerosis induction model used in the study of drugs for ischemic heart disease treatment.

In our experiment, once formed the plaque (6-8 months) in 16 rabbits, plasma samples were taken, and randomly assigned to treatment or placebo group. 4 months later new plasma samples were obtained. Therefore the experiment consisted of 32 samples corresponding to two groups at two different times: before and after receiving colchicine.

For the broadest metabolite coverage, a multiplatform metabolomics strategy was applied, based on GC/MS, CE/MS, and RP-HPLC/MS (positive and negative mode). Plasma fingerprints were pre-processed and the resulting matrixes analysed with different multivariate and bivariate analysis.

The results showed poor classification patterns of the multivariate metabolomics models. Nevertheless, by means of 2-way ANOVA and post-hoc analysis, it was possible to find metabolites with significant variations associated either to progression of the lesion, or to colchicine treatment, or both. Compounds such as urea and glycolic acid (from GC/MS), bile acids (from LC/MS(-) and LC/MS(+)), or kynurenine and proline-betaine (from CE/MS) showed different trends depending on the treatment.

The changes associated to lesion development and in the response to colchicine, will help to elucidate the metabolic processes involved in early stages of cardiovascular disease, as well as in pharmacological treatment of atherosclerosis.



**Poster #: 168**

**Abstract #: 2552**

**Abstract Title:** Inter-Sample Chemical shift variations in  $^1\text{H}$  NMR: An Improved Workflow for Measurement of Urine Metabolome and Biomarker Discovery

**Authors:** Ryan Gil,

**Presenting Author Affiliation:** HelmholtzZentrum München

**Abstract Submission:**

The metabolic profiling of human urine via nuclear magnetic resonance (NMR) spectroscopy has seen much advancement in recent years and become well established. Despite preparation techniques being relatively standardized the nature of human urine is highly variable and there is still room for improved protocols. In particular, patients with kidney disease and kidney failure can produce urine that is highly diverse in terms of pH and ionic strength as well as contain relatively high quantities of protein (i.e. proteinuria). These sample properties create what is known as 'positional noise' for metabolite peaks in NMR spectra. This interferes with the quality of multivariate statistical analysis outcome, thereby hindering the process of biomarker discovery. In order to optimize peak alignment and reduce positional noise, we apply a 2-dimensional buffering system, utilizing both potassium fluoride (KF) and phosphate buffers to reduce positional noise in metabolomic data from urine samples with varying levels of proteinuria. We show that the addition of KF reduces the mean inter-sample relative standard deviation (RSD) of citrate peaks of from 0.17 to 0.09. By reducing positional noise from ionic interferences with KF, multivariate statistical analysis such as statistical correlation spectroscopy (STOCSY) of citrate peaks saw significant improvement. We also further align spectral data using a recursive segment-wise peak alignment (RSPA) method, which leads to further improvement of the positional noise (RSD = 0.06) and a complete correlation of all citrate peaks via STOCSY. This workflow provides improved biomarker discovery for urine metabolomics of kidney disease patients. As quantification of metabolites is also critical, we applied quantification techniques to samples treated with, and without KF and observed positive results. This demonstrated that the addition of KF will not adversely affect metabolite quantification.

**Poster #: 169**

**Abstract #: 2454**

**Abstract Title:** Red blood cells metabolomics for the stratification of diabetic patients

**Authors:** Samia Boudah, Lydie Oliveira, Dhouha Darghouth, Paul-Henri Roméo, Benoit Colsch, Fabien Jourdan, Etienne Thévenot, Etienne Larger, Christophe Junot,

**Presenting Author Affiliation:** CEA

**Abstract Submission:**

The red blood cell (RBC) is the one of the simplest human cells. It has many transport proteins on its cell membrane, no nucleus, no cytoplasmic organelles and a life span of 120 days in humans. We have evaluated the relevance of red blood cells (RBC) metabolomics for the stratification of type 1 and type 2 diabetic patients, as an alternative to plasma for biomarker discovery.

Implementation of two liquid chromatography coupled to high resolution mass spectrometry methods and bioinformatic data processing tools led to the identification of 185 and 158 metabolites in plasma and RBC samples, respectively. Our results indicate that type 1 diabetic patients can be discriminated from type 2 patients on the basis of metabolic information. Forty six discriminating metabolites consisted of aminoacids and derivatives, carbohydrates, hydroxy acids, bile acid conjugates, uric acid and vitamins, were highlighted by statistical analyses performed on plasma metabolomic data. However, the concentrations of many of these metabolites (i.e., 29 out of 46) were impacted by physiological confounding factors such as age, gender and body mass index, or contextual factors such as on time glycemia, glycemia over 12 hours and glycated hemoglobin. Regarding RBC samples, our results show that concentrations of 22 metabolites were significantly different between type 1 and type 2 diabetic patients. Some of these metabolites are involved in glutathione metabolism, whereas some others are produced by the gut microbiota. Interestingly, our results show that RBC metabolomic data are far less impacted by physiological and contextual confounding factors than those of plasma.

In conclusion, RDB could be an interesting biological medium for biomarker discovery. Additional proof of concept studies are under progress in our laboratory for the stratification of type 2 diabetic patients and also in the field of chronic and inflammatory diseases such as rheumatoid arthritis.

**Poster #: 170**

**Abstract #: 2007**

**Abstract Title:** IMS-DIA-MS CHARACTERISATION AND IMS-MRM QCONCAT QUANTITATION OF THE LIPIDOME AND APOLIPOPROTEIN COMPLEMENTS OF OBESITY AND DIABETES COHORTS

**Authors:** Lee Gethings, Johannes Vissers, Jose Castro-Perez, Yvonne Woolerton, Lynn McLean, Robert Beynon, James Langridge,

**Presenting Author Affiliation:**

**Abstract Submission:**

Plasma samples were treated with isopropanol and centrifuged to precipitate proteins. 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 IMS oa-QToF platform using an ion mobility assisted data independent analysis acquisition workflow. Unsupervised MVA of the data showed clear distinction between cohorts. OPLS-DA was used to filter for features of significant correlation and covariance prior to identification. Identifications matching criteria were as follows, collision cross section (CCS) values 2 were considered for further interrogation. Ion mobility-derived CCS measurements allowed for improved specificity with the inclusion of drift time, providing additional confidence in the identifications returned. A variety of synthetic lipid standards representing the most significant classes identified were measured to determine their CCS for reference and used to populate the in-house database providing additional identification stringency. Normalized label-free quantitation results highlighted differential expression of specific lipid classes including fatty acids, phosphatidylcholines, triglycerides and phosphatidylserines. Additional identifications were obtained by mapping putative identifications with those from independent studies and biochemical networks. The identified networks were extended and confirmed by targeted IMS-MRM experiments of the most common alleles of the apolipoprotein plasma complement. Apolipoprotein AI, CI, CII, D, and E are key constituents of LDL and HDL and related to obesity, diabetes, and cardio vascular diseases, whereas Apolipoprotein E, a multifunctional protein, is also involved in lipid metabolism by mediating the binding of lipoproteins or lipid complexes to specific cell-surface receptors. The inclusion of IMS in the targeted oaToF MRM workflow increased quantitative precision and accuracy with the results in agreement with expected changes in relation with disease and/or phenotype.

**Poster #: 171**

**Abstract #: 2291**

**Abstract Title:** Metabolic profiling of bile acids in intestine and feces samples of diabetic mice using UPLC-MS

**Authors:** Nina Sillner, Alesia Walker, Wendelin Koch, Michael Witting, Philippe Schmitt-Kopplin,

**Presenting Author Affiliation:** Helmholtz Zentrum München, Deutsches Forschungszentrum

**Abstract Submission:**

A metabolic disorder such as Type2 Diabetes mellitus (T2DM) is a complex disease whereby genetic predisposition but also environmental and nutritional influences are discussed to be risk factors. The db/db mouse model, bearing a nonfunctional leptin receptor, is used to investigate T2DM. A pharmacological study was performed with db/db mice and their nondiabetic controls (wt) by administration of metformin for two weeks. High resolution mass spectrometry techniques were used for nontargeted analysis of intestinal and fecal samples to reveal metabolome patterns in diabetic mice, representing the interplay of the host metabolome and the gut microbiome. It was demonstrated that T2DM affects different metabolic pathways and their respective metabolites in the gastrointestinal tract. Especially bile acid and steroid metabolism were highly discriminative comparing the metabolomes of db/db and wt mice (Walker et al., J. Proteome Res. 2014). We assume that antidiabetic drugs, such as metformin, also alter bile acid metabolism. To investigate this, an absolute quantification method by ultra-performance liquid chromatography-mass spectrometry (UPLC-MS) for targeted bile acid profiling in different biological matrices was developed and validated according to FDA guidelines.

**Poster #: 172****Abstract #: 2319****Abstract Title:** Application of capillary electrophoresis mass spectrometry for unravelling changes associated with gestational diabetes mellitus**Authors:** Danuta Dudzik, Antonia García, Mariusz Skotnicki, Marcin Zorawski, Wieslaw Zarzycki, Santiago Angulo, Coral Barbas,**Presenting Author Affiliation:** CEMBIO (Center for Metabolomics and Bioanalysis),**Abstract Submission:**

Gestational diabetes mellitus (GDM) is defined as any degree disturbance of glucose metabolism with onset or first recognition during pregnancy. Despite its short duration GDM has long term adverse consequences both for mother and baby. Many studies confer, that women with GDM are at higher risk of subsequent type 2 diabetes, metabolic syndrome and cardiovascular disease development later in life.

Nowadays, untargeted metabolomics offers a powerful tool for identification of key metabolites involved in the biological or pathophysiological processes at a molecular level. That, allow for new insight and the elucidation of disease mechanisms, onset and progression. In our study we applied capillary electrophoresis coupled to electrospray ionization - time of flight - mass spectrometer (CE-TOF-MS) for comprehensive analysis of polar and ionic metabolites related to gestational diabetes.

The study sample consisted of 64 participants - 36 healthy pregnant women and 28 women with GDM (2-h 75-g OGTT) matched according to week of gestation and age were collected following gestational and postpartum time trajectory (2nd, 3rd trimester of gestation, 1 month and 3 months after delivery). An Agilent Technologies 7100 CE system coupled to an Agilent Technologies 6224 Accurate-Mass TOF mass spectrometer system with an electrospray source was used for metabolomics analysis.

Comprehensive data mining including data pre-processing, data pre-treatment and data treatment have been applied to the study. A data matrix consisting of 135 metabolic features were evaluated by univariate and multivariate statistical analysis. The metabolic perturbation identified in this study, mainly related to amino acids and carnitine pathways, may provide an important further insight into the molecular pathophysiological mechanism of the onset and progression of GDM.

**Poster #: 173**

**Abstract #: 2526**

**Abstract Title:** Metabolic profiling of human aorta with atherosclerotic plaques using liquid chromatography/mass spectrometry.

**Authors:** Sunhee Jung, Miso Nam, Do Hyun Ryu, Geum-Sook Hwang,

**Presenting Author Affiliation:** Sungkyunkwan University

**Abstract Submission:**

Atherosclerosis is a chronic inflammatory disease characterized by thickening of the arterial wall resulting from abnormal lipid accumulation. However, only a limited number of studies on atherosclerosis was proceeded in metabolic profiling of human tissue. In the present study, we performed the global profile of aorta with plaques and aorta without plaques of human using ultra-performance liquid chromatography/quadrupole time-of flight mass spectrometry (UPLC/Q-TOF MS). Principal component analysis (PCA) plots and partial least squares-discriminant analysis (PLS-DA) plots obtained from polar extracts and non-polar extracts of aorta tissue showed a clear differentiation between aorta with plaque and aorta without plaque. Significantly altered metabolite species were amino acid, purine, pyrimidine, and choline. In addition, lipid metabolites including long chain-ceramide (Cer), lysophosphatidylcholine (lysoPC), phosphatidylcholine (PC), phosphatidylethanolamines (PE), phosphatidylinositol (PI), sphingomyelin (SM), diacylglycerol (DG), and triacylglycerol (TG) species were elevated in aorta with plaque. Particularly, purine, pyrimidine, Cer, and lysoPC which were metabolites in well-established pathway in atherosclerosis were highly changed in aorta with plaque. This study demonstrates that UPLC/Q-TOF MS based global profiling can be useful tool to understand metabolite distribution of human atherosclerotic aorta and may provide the insight for pharmacotherapeutic intervention.

**Poster #: 174**

**Abstract #: 2555**

**Abstract Title:**  $^1\text{H}$  NMR-based metabolomic profiling reveals potential biomarkers of renal function in non-diabetic and diabetic populations

**Authors:** Jonas Zierer, Clara Barrios, Gabi Kastenmüller, Sol Otero, Eva Rodríguez, María José Soler, Tim Spector, Julio Pascual, Cristina Menni,

**Presenting Author Affiliation:** Dept. for Twin Research, King's College London

**Abstract Submission:**

Chronic kidney disease is an increasing public health problem, which is partially a consequence of the higher incidence of type 2 diabetes. Traditional biomarkers of renal function, such as creatinine, typically indicate disease only when kidney function is already substantially decreased. We aim to identify human blood metabolites associated with renal function, particularly in early stages of disease.

We applied a non-targeted metabolomics approach using  $^1\text{H}$  NMR to determine absolute concentrations of 147 serum metabolites in 1851 subjects from the TwinsUK cohort. We analysed correlations with renal function, measured as estimated glomerular filtration rate (eGFR), using linear mixed models adjusting for covariates (age, sex, BMI and family structure) and multiple testing (Bonferroni threshold  $p < 3.4 \times 10^{-4}$ ). Significant metabolites were replicated in 445 diabetic subjects from the Genodiab-Mar cohort. Results from both cohorts were then meta-analysed.

Fifteen metabolites were associated with renal function and replicated in the diabetic cohort. Even though none of the classic blood lipid measures was associated with eGFR in our cohorts, we found two triglyceride subclasses negatively and four HDL subclasses positively associated with renal function. Interestingly, we also found the inflammatory marker  $\alpha 1$ -acid glycoprotein acetyls ( $\beta = -4.60$  [-6.38:-2.82]), glycine ( $\beta = -3.79$  [-4.96:-2.61]) and citrate ( $\beta = -7.09$  [-8.65:-5.53]), which have been previously associated with cardiovascular risk and mortality and which are also associated with fat mass and blood glucose levels in the TwinsUK cohort, independently of eGFR. Moreover increased abundance of phenylalanine, which was previously suggested to be involved in early renal damage, was associated with decreased renal function ( $\beta = -6.58$  [-8.58:-4.59]). However, we did not find significant correlations of phenylalanine with other diabetes-related symptoms, suggesting a more direct association with renal function.

We identify find several novel markers of renal function, consistent in a healthy and a disease cohort, which might help to identify common mechanisms of diabetic and non-diabetic kidney disease.

**Poster #: 175**

**Abstract #: 2056**

**Abstract Title:** Serum metabolic disturbances associated with acute myocardial infarction elucidated by NMR-based metabolomics

**Authors:** Atul Rawat, Dinesh Kumar,

**Presenting Author Affiliation:** CBMR/BBAU

**Abstract Submission:**

**Background:** Acute myocardial infarction (AMI) is the leading cause of cardiac-related deaths worldwide. The traumatic and devastating outcome is sudden death of the patient within first few hours from the onset of symptoms. The rapid detection of physiological transformations associated with AMI coupled with instant treatment to reset these changes and monitoring response to treatment can greatly decrease the mortality and morbidity of patients.

**Methodology:** Metabolic profiles of sera collected from 42 AMI patients (immediately after the myocardial infarction) and 40 normal controls were obtained using high-resolution 1D 1H CPMG and diffusion edited NMR spectra. The metabolic profiles were compared using multivariate statistical analysis to identify the metabolic disturbances associated with AMI and, therefore, the perturbed biochemical pathways in this condition.

**Results:** The sera of AMI patients were characterized (a) by the increased levels of arginine, proline, glucose, glycerol, myoinositol, ornithine, creatine, cholesterol, LDL/VLDL, N-acetyl glycoproteins (NAG), and lipid and (b) by the decreased levels of HDL, unsaturated lipids, albumin-lysyl, lactate, pyruvate, acetoacetate, choline, and most of the amino acids. The observed metabolite perturbations in the sera of AMI patients were involved in multiple metabolic pathways, associated with dyslipidemia, necrosis and profoundly dampened glycolysis, Krebs cycle, fatty acid  $\beta$ -oxidation, and amino acid metabolism, alluding to reduced energy biogenesis from all sources.

**Conclusion:** Significant serum metabolic perturbations were detected in AMI patients compared to normal control suggesting the feasibility of NMR-based metabolomics for rapid diagnosis and clinical management of myocardial infarction in future clinical settings.



**Poster #: 176**

**Abstract #: 2103**

**Abstract Title:** Cord blood metabolome is associated with birth weight, but not predictive for weight gain and later BMI

**Authors:** Olaf Uhl, Christian Hellmuth, Marie Standl, Joachim Heinrich, Elisabeth Thiering, Berthold Koletzko,

**Presenting Author Affiliation:** LMU Munich

**Abstract Submission:**

Obesity and its consequences such as insulin resistance are an increasing challenge for developed countries. Several studies have discovered a list of biomarker for obesity and insulin resistance in adults but detailed studies are missing considering children or infants. However, these studies are relevant to understand the inter-generational transfer of obesity and the pathology of obesity-related comorbidities with aim to find new intervention targets in early life periods. The objective of the presented work is to depict associations of cord blood metabolites with birth weight, postnatal weight gain, and later BMI.

A targeted analytical platform was used for the quantification of metabolites in 753 cord blood samples from the German birth cohort study LISAplus. Cord proteins were precipitated by adding methanol including internal standards. After centrifugation, the supernatant was split into aliquots for the analyses of individual methods detecting polar lipids, acylcarnitines, amino acids and non-esterified fatty acids (NEFA) by liquid chromatography triple quadrupole mass spectrometry analyses.

Glycerophospholipid fatty acids (GPL-FA) were measured by gas chromatography after derivatization to fatty acid methyl ester. Associations between metabolite concentrations in cord blood to birth weight, weight gain and BMI at 2 and 15 years of age were determined using linear regression models adjusted for confounding variables.

In total, 581 metabolites were measured of which 209 passed the quality control procedures (CV<30%). Several positive associations between birth weight were found to lyso-phosphatidylcholines LPC(16:1), LPC(18:1), LPC(20:3), LPC(18:2), LPC(20:4), LPC(14:0), LPC(16:0), LPC(18:3), GPL-FA(20:3n9), and GPL-FA(22:5n6), while negative associations were found to NEFA(22:6), NEFA(20:5), GPL-FA(18:3n3) and PCe(38:0). In contrast, postnatal weight gain and BMI in adolescents, no significant associations were observed after adjustment for multiple testing.

Thus, cord blood metabolites are highly associated with birth weight and reflect the intra-uterine development, but have less potential to predict patterns of later weight and growth.

**Poster #: 177****Abstract #: 2197**

**Abstract Title:** Indolepropionic acid and certain lipid metabolites are related to decreased risk of developing type 2 diabetes in the Finnish Diabetes Prevention Study

**Authors:** Kati Hanhineva, Vanessa de Mello, Jaana Lindström, Maria Lankinen, Jussi Paananen, Johanna Kuusisto, Jussi Pihlajamäki, Seppo Auriola, Marko Lehtonen, Rikard Landberg, Elise Nordin, Jaakko Tuomilehto, Matti Uusitupa,

**Presenting Author Affiliation:** University of Eastern Finland

**Abstract Submission:**

The Finnish Diabetes Prevention Study (DPS) was a randomized, controlled, multicenter study involving lifestyle intervention aiming at reduction of type 2 diabetes (T2D) in people with impaired glucose tolerance. We applied the non-targeted metabolite profiling analytics utilizing mass spectrometry combined to liquid chromatography with two chemistries reversed phase and hydrophilic interaction chromatographies on fasting plasma samples collected at year 1 after randomization from 200 participants, who either developed T2D within 5-year follow-up (n=96) or did not convert to T2D within 15-year follow-up (n=104). We observed that several metabolites were associated with lower likelihood of developing T2D (FDR-P < 0.05). The results showed that higher plasma levels of the gut microbiota derived compound indolepropionic acid were related to lower likelihood of developing T2D, and additionally, higher levels of indolepropionic acid predicted a better insulin secretion capacity and thus may have protective effect on the beta cell function. This finding was associated with the increased intake of whole grain products, confirmed by alkylresorcinol biomarkers for whole grain intake which were positively associated with indolepropionic acid level. Additionally, another novel finding in our study was that several lipid compounds were highly associated with reduced risk of T2D. Common for many of these lipids was that they contained fatty acids with odd carbon number. Interestingly, our preliminary analyses indicate that the occurrence of these lipids is associated with inflammation related biomarkers, as the protective lipids identified as lysophosphatidylcholines were all inversely correlated with high-sensitivity C-reactive protein ( $r=0.37-0.43$ ,  $P<0.000001$ ), and therefore may hold a role in the modulation of the inflammatory responses related to obesity and T2D. Moreover, several aromatic and branched chain amino acids were associated with an increased likelihood of developing T2D (FDR-P<0.05), which confirms earlier metabolic findings related to development of T2D.

**Poster #: 178**

**Abstract #: 2400**

**Abstract Title:** NMR-based assessment of liraglutide therapy effectiveness

**Authors:** Martina Bugánová, Helena Pelantová, Martina Holubová, Blanka Šedivá, Blanka Železná, Lenka Maletínská, Jaroslav Kuneš, Marek Kuzma,

**Presenting Author Affiliation:** Institute of Microbiology ASCR

**Abstract Submission:**

Nowadays, a large spectrum of antidiabetic drugs are available on the market. While metformin is a first-choice agent in the treatment of type 2 diabetes mellitus (T2DM), the suitable alternative drugs are still intensively discussed. Accordingly, novel approaches are required to determine the effectivity of the various antidiabetic drugs even at early stages of the administration.

In our work we show that urine metabolomics studied by NMR spectroscopy in mouse model of diet-induced obesity and T2DM is a promising method for the tracking of the antidiabetic therapy effectiveness. We investigated the changes in urinary metabolic profiles of obese mice induced by the two-week therapy by liraglutide, which is an antidiabetic agent with additional weight reducing effect. The positive impact of liraglutide was proved by significant decrease of blood glucose concentrations, body weight and leptin levels. Metabolomic approach captured several changes in metabolites in urine of obese and diabetic mice as a result of the treatment the significant decrease was found in the levels of nicotinamide metabolites (N-methyl-2-pyridone-5-carboxamide, N-methyl-4-pyridone-3-carboxamide, and trigonelline), which have already been reported in connection with obesity and T2DM. The concentrations of N-carbamoyl- $\beta$ -alanine, cis-aconitate, glucose, succinate and taurine were also significantly diminished. Contrary, an increase was observed in levels of 3-indoxylsulfate, dimethylglycine and phenylacetyl-glycine. Finally, a strong correlation was found among the levels of the significantly changed urinary metabolites and the body weight, the blood glucose levels and the amount of adipose tissues.

Altogether, our study indicates that NMR-based metabolomic analysis of urine can substantially extend the information on the liraglutide therapy effectiveness obtained by the standard biochemical methods. This information could be then potentially utilized in clinical practice.

**Acknowledgements:** This work is supported by the Grant Agency of the Czech Republic (Grant No. 13-14105S) and the Operational Program Prague – Competitiveness (Project No.: CZ.2.16/3.1.00/24023).

**Poster #: 179**

**Abstract #: 2401**

**Abstract Title:** HRMS and MRM based screening - where to start in Clinical Metabolomics

**Authors:** Roland Geyer, Alexander Leichtle, Christos Nakas, Michael Hayoz, Anke Toenjes, Martin Fiedler,

**Presenting Author Affiliation:** INSELSPITAL - University Hospital Bern, University

**Abstract Submission:**

Blood samples of a sorbian population were obtained 30 and 120min after intervention by an 75g oral glucose tolerance test. The serum samples were prepared with a commercially available kit to extract acylcarnitines, glycerophospho- and sphingolipids as well as amino acids. Eluates were analyzed by triple quadrupole mass spectrometry with chromatographic separation and by high resolutions mass spectrometry using the same chromatography. Peak picking and alignment was done either with Progenesis Q1 software (Waters) or MRM-DIFF, a data processing tool for multiple reactions monitoring (MRM)-based differential analysis. The different sets of raw data were transposed and imported in SIMCA (Umetrics) to enable a comparison by multivariate statistical analysis methods (e.g., PCA, OPLSD-DA). Scripts from the r-project were used for a paired test design (multilevel sPLS-DA).

Based on a 12min chromatographic separation of 111 mass transitions covering glycerophosphatidylcholine- and sphingolipids in positive electrospray mode more than 1800 peaks were extracted, while scanning with quadrupole time-of-flight (QToF) HRMS revealed about 4400 features. Peaks from the semi-targeted or non-targeted analysis were annotated by their retention time and mass or by mass transition. After data clean up QToF data showed about 3 times more features (~800 vs 2300, respectively). However, neither principal component analysis (PCA) nor OPLS-DA algorithm could clearly separate samples sets for the two time points as inter-individual variability was higher than the metabolic effects of concentrated glucose intake. Using a paired test design with multilevel sPLS-DA algorithm the two time points were clearly separated based on both data sets.

As specificity for the MRM analysis is higher but data amounts and analysis time much smaller compared to full scan HRMS peak data the use of mass transitions may be more efficient to start with for an initial grouping and identifying outliers in clinical metabolomics sample sets.

**Poster #: 180**

**Abstract #: 2556**

**Abstract Title:** Lipid metabolism in hypothalamic neuron treated with saturated fatty acid using high resolution mass spectrometry

**Authors:** Sandrine Aros-Calt, Mélanie Campana, Christophe Magnan, Christophe Junot, Hervé Le Stunff, Benoit Colsch,

**Presenting Author Affiliation:** MedDay Pharmaceuticals

**Abstract Submission:**

Recent studies have shown that accumulation of some lipid species, such as ceramides, in the hypothalamus would be responsible in part of a central nervous system's lipotoxicity. Indeed, this could play a role in the onset of insulin resistance and development of type 2 diabetes by deregulation of the nervous control of glucose homeostasis. In the present study, we have been explored the effect of saturated fatty acids on lipid metabolism in hypothalamic neurons using an untargeted lipidomic approach.

Palmitate (C16:0), a precursor of ceramide biosynthesis, was added to culture medium containing hypothalamic cell line GT1-7 at different exposure times and compared with bovine serum albumin (BSA) as culture medium control. The total lipid extract of hypothalamic cells was analyzed by reverse phase liquid chromatography coupled to high resolution mass spectrometry (RPLC/HRMS) using a Q-Exactive Plus instrument. Automatic peak detection was performed using the XCMS software whereas lipids identification was achieved using in-house lipid database and MS/MS experiments.

Regarding to BSA controls, a significant increase of total ceramide species, particularly C16:0 and C18:0 lipid species was observed. In order to highlight the metabolic pathways impacted by the addition of palmitate, a deuterated palmitate was added in the same culture conditions as described previously. To annotate produced deuterated lipid species, the in-house lipid database was implemented with d4 and d8 lipids species. This global lipidomic approach allowed a simultaneous detection of endogenous and deuterated ceramides species but also other lipid families in the deuterated forms. Diagnostic ions of deuterated sphingosine and palmitate in both ionization modes were confirmed by MS/MS experiments (using higher energy collisional dissociation (HCD)). These results showed an incorporation of deuterated palmitate in the "de novo" sphingolipid biosynthesis and in other lipid pathways. In future studies, an inhibitor of sphingolipid biosynthesis will be added to confirm these results.

**Poster #: 181**

**Abstract #: 2385**

**Abstract Title:** Structural equation modeling: Linking exposure to birth- and early life-health outcomes via the metabotype of cord blood

**Authors:** Susan McRitchie, Susan Sumner, Andrea Richardson, Wimal Pathmasiri, Frederica Perera,

**Presenting Author Affiliation:** RTI International

**Abstract Submission:**

While maternal exposure to polycyclic aromatic hydrocarbons (PAH) has been linked to birth- and early-life health outcomes, the mechanisms of action are not well understood. We used metabolomics to analyze cord blood serum obtained from a cohort established at the Columbia Center for Children's Environmental Health, and used structural equation modelling (SEM) to link perturbations in linear combinations of metabolites in cord blood, with measures of prenatal exposure, birth outcomes, and measures of the infant's cognitive development at 12, 24, and 36 months of age. Prenatal exposure to PAHs was characterized by the measurement of PAHs and PAH-derived metabolites in maternal serum and urine, and cord blood benzo[a]pyrene-DNA adducts. SEM demonstrated significant pathway associations between the exposure markers, the cord blood metabotype, and birth head circumference, birth weight, and cognitive development. This application of SEM and pathway analysis to relate prenatal exposures to health outcomes via the cord blood metabotype provides a means to reveal pathways and mechanisms important to birth and early life outcomes associated with prenatal exposure. The metabolomics analysis was funded by the NIH Common Fund Metabolomics Program (1U24DK097193, Sumner, PI), and the human subject investigations and exposure assessments (Perera, PI) were funded by New York Community Trust, NIEHS, and the US EPA: NIEHS/EPA P01ES09600/R82702701, NIEHS/EPA P01ES09600/RD83214101, NIEHS/EPA P01ES09600/RD83450901, and NIEHS R01ES08977.

**Poster #: 182**

**Abstract #: 2475**

**Abstract Title:** Profiling of plasma metabolites in postmenopausal women with metabolic syndrome

**Authors:** Miho Iida, Sei Harada, Ayako Kurihara, Kota Fukai, Ayano Takeuchi, Tomonori Okamura, Akiyoshi Hirayama, Masahiro Sugimoto, Tomoyoshi Soga, Masaru Tomita, Toru Takebayashi,

**Presenting Author Affiliation:** Keio University

**Abstract Submission:**

[Objective] To investigate the associations of amino acids and other polar metabolites with metabolic syndrome in postmenopausal women in a lean Asian population.

[Methods] The participants were 1422 female residents enrolled in a cohort study from April to August 2012. Metabolic syndrome was defined according to the National Cholesterol Education Program Adult Treatment Panel III modified for Japanese women. Associations were examined between metabolic syndrome and 78 metabolites assayed in fasting plasma samples using capillary electrophoresis mass spectrometry. Replication analysis was performed to confirm the robustness of the results in a separate population created by random allocation.

[Results] Analysis was performed for 877 naturally postmenopausal women, including 594 in the original population and 283 in the replication population. The average age, body mass index, and levels of high- and low-density lipoprotein cholesterol of the entire population were 64.6 years old, 23.0 kg/m<sup>2</sup>, 72.1 mg/dl and 126.1 mg/dl, respectively. There was no significant difference in low-density lipoprotein cholesterol levels between women with and without metabolic syndrome. Thirteen metabolites were significantly related to metabolic syndrome: multiple plasma amino acids were elevated in women with metabolic syndrome, including branched-chain amino acids, alanine, glutamate and proline and alpha-amino adipate, which is generated by lysine degradation, was also significantly increased.

[Conclusions] Our large-scale metabolomic profiling indicates that Japanese postmenopausal women with metabolic syndrome have abnormal polar metabolites, suggesting altered catabolic pathways. These results may help to understand metabolic disturbance, including in persons with normal BMI and relatively high levels of HDL-C, and may have clinical utility based on further studies.

**Poster #: 183**

**Abstract #: 2480**

**Abstract Title:** Metabolic flexibility in the insulin resistant heart and skeletal muscle: a dynamic <sup>13</sup>C labelling approach

**Authors:** David De Souza, Greg Kowalski, Sean O'Callaghan, Joachim Kloehn, Dedreia Tull, Malcolm McConville, Clinton Bruce,

**Presenting Author Affiliation:** Metabolomics Australia, The University of Melbourne

**Abstract Submission:**

**Rationale:** Cardiac and skeletal muscle metabolism is thought to be altered in insulin resistance and type 2 diabetes (T2D). Our understanding of the regulation of metabolism and insulin sensitivity has largely been derived from ex vivo preparations which are not subject to the same metabolic regulation as in the intact heart in vivo. Studies are therefore required to examine in vivo glucose metabolism under physiologically relevant conditions.

**Objective:** To determine the temporal pattern of the development of cardiac and muscle insulin resistance and to compare with dynamic approaches to interrogate cardiac glucose and intermediary metabolism in vivo.

**Methods and results:** Studies were conducted to determine the evolution of cardiac and skeletal muscle insulin resistance in C57Bl/6 mice fed a high-fat diet (HFD) for between 1 and 16 weeks. Dynamic in vivo cardiac glucose metabolism was determined following oral administration of [U-<sup>13</sup>C] glucose. Hearts and skeletal muscle were collected after 15 and 60 min and flux profiling was determined by measuring <sup>13</sup>C mass isotopologues in glycolytic and tricarboxylic acid (TCA) cycle intermediates. Cardiac insulin resistance, determined by euglycemic-hyperinsulinemic clamp, was evident after 3 weeks of HFD. Despite the presence of insulin resistance, in vivo cardiac glucose metabolism following oral glucose administration was not compromised in HFD mice. This contrasts our findings in skeletal muscle, where TCA cycle activity was reduced in mice fed a HFD. Similar to our findings in skeletal muscle, glucose derived pyruvate entry into the TCA cycle in the heart was almost exclusively via pyruvate dehydrogenase, with pyruvate carboxylase mediated anaplerosis being negligible after oral glucose administration.



**Poster #: 184**

**Abstract #: 2161**

**Abstract Title:** Effects of glucocorticoids administration and their interaction with insulin: an untargeted metabolomics investigation

**Authors:** Riccardo Di Guida, Warwick Dunn, Jonathan Hazlehurst, Jeremy Tomlinson,

**Presenting Author Affiliation:** School of Biosciences, University of Birmingham

**Abstract Submission:**

Introduction: Metabolomics is a powerful tool for the characterisation of human diseases or physiological traits that are yet to be completely understood. Glucocorticoids are largely used in pharmacological interventions targeting specific mechanisms or pathways. However, their global effects on metabolism are not completely understood. Whilst they provide an anti-inflammatory effect, they also influence metabolic pathways including lipogenesis and gluconeogenesis in a tissue-specific manner. It was estimated that 1-2% of the population of the United States and United Kingdom are prescribed glucocorticoids therapy<sup>1</sup>.

Objectives and Methods: To shed light on the metabolic perturbations observed in conjunction with glucocorticoids treatment, two studies involving untargeted metabolomics and the use of UPLC-MS techniques were performed. Study one was performed on human serum samples and focused on the effects of cortisol administration and separately on the effects of cortisol and insulin treatment. Study two involved the assessment of the effects of two 5- $\alpha$  reductase inhibitors and their interaction with insulin. 5- $\alpha$  reductase is an enzyme with an essential role in steroid biosynthesis including the conversion of testosterone into dihydrotestosterone, hence its inhibition is a common target in pharmacological therapies.

Results and conclusions: Study 1 showed that glucocorticoids and insulin perturb the same metabolic pathways in either a cooperative or inhibitive manner. This study highlighted a possible suppression by insulin on the metabolic changes caused by cortisol administration and an involvement of insulin in activation of lipogenic pathways. Study 2 showed a negating influence on the metabolic effects of insulin operated by the two inhibitors while small global metabolic changes were induced by the inhibitors alone. Moreover, insulin administration induced a larger global metabolic effect when compared with the two inhibitors.

1. Hazlehurst, J.M., et al. Glucocorticoids fail to cause insulin resistance in human subcutaneous adipose tissue in vivo. *J Clin Endocrinol Metab* 98, 1631-1640 (2013).

**Poster #: 185**

**Abstract #: 2175**

**Abstract Title:** GC/MS based steroid profiling method for rat serum with large volume injection

**Authors:** Udi Jumhawan, Toshiyuki Yamashita, Motonao Nakao, Kuniyo Sugitate, Takeshi Serino, Ryoichi Sasano, Yoshihiro Izumi, TAKESHI BAMBA,

**Presenting Author Affiliation:** Kyushu University

**Abstract Submission:**

Research on steroidogenesis has recently attracted widespread interest due to extensive exposure of potential endocrine-disrupting chemicals (EDC). This group of chemicals can alter endocrine functions and ultimately cause hostile health effects. Recently, conventional immunological approaches to define steroid disorder are progressively replaced concomitantly with the surging application of mass spectrometry-based analysis. Our group has previously constructed a sensitive steroid analysis system for in vitro cell culture screening method employing gas chromatography/tandem mass spectrometry (GC/QqQMS) configured to the Multiple Reaction Monitoring (MRM) mode. We have further developed analytical system for simultaneous and precise measurement of steroid hormones in rat serum.

Serum is among the common biological samples used for steroid analysis, and due to 'noisy' background of the samples, matrix effect has been one of the major challenges. Furthermore, trace level of steroids in biological fluids and the existing isomers can hinder the detection and identification of steroids. We employed SPE and cleanup protocol to reduce complexity of the sample matrix and increase recovery. A 10  $\mu$ L of serum extract was injected using large volume injection protocol via unique GC insert. In this novel method, we set up solvent vent system enabling samples pre-concentration prior to entering column. Additionally, self-cleaning ion source system that introduces constant H<sub>2</sub> flow into the source was installed to enhance MS performance and minimize maintenance. Preliminary experiment of targeted GC/MS/MS analysis showed successful detection of steroid hormones actively involved in steroidogenesis. The result presents comprehensive coverage in terms of the number of detected steroids. Estimate concentrations of steroid showed comparable levels with previous rat serum studies. Development of analytical system for simultaneous steroid analysis serves as essential step to establish a high throughput evaluation of EDC that provides wealth information related to EDC-induced steroidogenesis disorder.

**Poster #: 186**

**Abstract #: 2178**

**Abstract Title:** Workflow methodology for rodent brain metabolome exploration using NMR, LC-HRMS and GC-MS analytical platforms

**Authors:** Binta DIEME, Antoine LEFEVRE, Lydie NADAL-DESBARATS, Laurent GALINEAU, Frederic MONTIGNY, Helene BLASCO, Christian ANDRES, Patrick EMOND, Sylvie MAVEL,

**Presenting Author Affiliation:** Université François-Rabelais, INSERM U930 "Imageri

**Abstract Submission:**

We developed a multiplatform approach for the metabolome exploration of rodent brain tissue using nuclear magnetic resonance spectroscopy (NMR), gas-chromatography coupled with mass spectrometry (GC-MS) and liquid chromatography coupled with high resolution mass spectrometry (LC-HRMS). The main steps for the widest and deepest metabolite exploration of cerebral tissues are lysis and metabolite extraction. We first analyzed the impact of freeze-drying on tissue and, four extraction methods to extract tissue metabolites were compared. The samples were analyzed by NMR and LC-HRMS using reversed phase liquid chromatography. Based on the number of metabolites extracted and their coefficient of variation (%CV), the most reproducible protocol (one-step extraction with acetonitrile on lyophilized material) was chosen to further test the impact of sample mass on method performance (3, 6, and 9 mg were tested). GC-MS analysis was also investigated using four different methoximation/silylation derivatization combinations. The optimal analytical protocols, for the three analytical platforms, were determined to establish the reliability and suitability of sample treatments required to achieve the largest untargeted brain tissue metabolomics analysis. An example of the application of this workflow by analyzing three cerebral rodent brain regions (n=3x4) by 1H NMR, GC-MS and LC-HRMS was presented, and the different cerebral regions were distinguished based on their metabolic profiles

**Poster #: 187**

**Abstract #: 2206**

**Abstract Title:** POTENTIAL OF METABOLIC PROFILING BY UHPLC-MS IN A CLINICAL LABORATORY

**Authors:** Cédric Bovet,

**Presenting Author Affiliation:** Inselspital, Bern University Hospital

**Abstract Submission:**

Nowadays, ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) achieves broad metabolome coverage in biological matrices. For a clinical laboratory, combining UHPLC-MS with the large set of clinical assays could accelerate the discovery and validation of metabolic signatures.

To assess this potential, we established a complete analytical workflow for the metabolic profiling of plasma, serum, urine and tissue by high-resolution UHPLC-MS (UPLC coupled to Synapt G2-S HDMS Q-TOF, Waters). The broad polarity of metabolites can be assessed by reversed-phase liquid chromatography (RPLC) and hydrophilic interaction liquid chromatography (HILIC). By using RPLC, we successfully discovered exercise-related metabolic differences between intermittent high intensity exercise (IHE) and iso-energetic continuous moderate intensity exercise (CONT) in individuals with well-controlled type 1 diabetes. Discriminant analysis of the 2534 detected features in serum suggested that the purine metabolism, cortisol and acylcarnitines were significantly regulated at 80 min of exercise and 120 min post-exercise compared to baseline with significant differences between IHE and CONT. The variations in the profiles of metabolites were then validated with clinical assays routinely used in our laboratory. Cortisol and uric acid (end product of purine degradation) were quantified on a clinical chemistry analyzer (Cobas 8000, Roche), acylcarnitines on a triple quadrupole mass spectrometer (UPLC coupled to Xevo TQ-S, Waters). Our results demonstrated that clinical laboratories could benefit from the synergy existing between UHPLC-MS and conventional clinical assays. Their combination offers an efficient panel for discovering regulated pathways, thereby strengthening multidisciplinary collaborations and translational research.

**Poster #: 188**

**Abstract #: 2201**

**Abstract Title:** Application of mass spectrometry-based lipidomics in the study of bipolar disorder

**Authors:** Alessandra Sussulini, Henrique Ribeiro, Aline Klassen, Caroline Dal Mas, Maiara Zeni-Graiff, Sumit Sethi, Mirian Hayashi, Quirino Cordeiro Junior, Elisa Brietzke,

**Presenting Author Affiliation:** Department of Analytical Chemistry, UNICAMP

**Abstract Submission:**

Bipolar disorder is considered one of the most harmful types of psychiatric pathologies, involving cognitive, neurochemical, psychological, socioaffective, and functional aspects. With a relatively high predominance in the adult world population (about 4%, independently of race or gender), this disorder is associated to cognitive function and psychosocial impairments observed in the patients and presents a high mortality rate. Studies that can help in the diagnosis, as well as the comprehension of the biochemical alterations related to the disorder, are of great interest and the search for biomarkers is a key strategy. Biomarkers are defined as indicators of a disease that can be efficiently measured without high costs, preferentially using non-invasive methods and be associated with the target illness. This work aims the analysis of the lipidomic profile of blood serum samples from bipolar disorder patients compared to healthy controls in order to search for potential biomarkers for this specific disorder. The methodology comprised the lipid extraction from serum samples followed by analysis by ultra-performance liquid chromatography coupled to mass spectrometry (UPLC-ESI-QTOF MS). Acquired data were then submitted to a pretreatment with the software XCMS followed by a multivariate analysis using the software SIMCA 14 to obtain the discriminant features that were subsequently identified by searches in lipid databases. With the results obtained so far, we were able to detect statistically significant differences between bipolar and control groups, being 116 features determined with the higher level in control groups and 108 features determined with the higher level in bipolar groups. Fatty Acyls (FA) and Glycerophospholipids (GP) were the most relevant features for discriminating these groups. This information can guide us in the forthcoming identification of differential molecules that can be potential biomarkers of bipolar disorder, helping in the diagnosis of this illness, which even nowadays is still limited to medical observations.

**Poster #: 189**

**Abstract #: 2469**

**Abstract Title:** Serum metabolomics study for identification of potential biomarkers of long-term survival in renal transplantation patients based on liquid chromatography-tandem mass spectrometry

**Authors:** Bo Kyung Kim, Yong Chul Shin, Mi-Ri Gwon, Moonyoung Jegal, Sook Jin Seong, Jang-Hee Cho, Chan-Duck Kim, Young-Ran Yoon,

**Presenting Author Affiliation:** Kyungpook National University

**Abstract Submission:**

The recent progress and appropriate use of immunosuppressive drugs have considerably improved the outcome of short-term survival in renal transplantation patients. In addition, the development of new strategies to improve long-term survival outcome after renal transplantation is also becoming important. Although current diagnosis of renal transplantation relies on serum creatinine concentrations, oliguria, anuria and biopsy, they are nonspecific indicators of renal function. Therefore, noninvasive, sensitive, and specific biomarkers for the prediction of long-term survival are needed. The aim of this study is to discover potential biomarkers for long-term survival in renal transplantation patients using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

We used the metabolic approach to explore the change of metabolites in the serum of renal transplantation patients. Fifty four patients were participated in this study and serum samples were collected on the basis of their medical history and routine clinical laboratory test. After quantile normalization with chromatographic data, multivariate statistical analysis was performed using SIMCA 14.

We attempted to analyze metabolic profiling with long-term survival and chronic renal failure groups. Orthogonal partial least squares discriminant analysis (OPLS-DA) score plot showed a separation between two groups in the principal component. In the corresponding loading plot, the metabolite responsible for the separation observed in the score plot were identified (VIP = 1.0).

We will further examine the additional test for more sensitive and accurate analysis and finally we will figure out potential biomarkers for the prediction of long-term survival in renal transplantation patients.

**Poster #: 190****Abstract #: 2534****Abstract Title:** Improving the metabolic stability of cultured cells during extended HR-MAS NMR measurements by prior heating**Authors:** Gaëlle Diserens, Damian Hertig, Balazs Legeza, Sandra Eggimann, Martina Vermathen, Julien Furrer, Christa Flück, Jean-Marc Nuoffer, Peter Vermathen,**Presenting Author Affiliation:** DCR & DIPR, Bern University**Abstract Submission:****Introduction:**

<sup>1</sup>H-HR-MAS NMR is an established tool for metabolic characterization of biological samples. However, the accuracy of biomarker detection depends on the sample stability over measurement time. Previously, Duarte et al. found enhanced metabolite visibility and different lipid profiles in lysed compared to frozen cells [1]. Here we investigated effects of short-time heating prior to HR-MAS measurements on metabolite stability of cells. We hypothesized that cell heating has only minor effects on initial metabolite contents (i.e. initially similar spectra with and without heating) and results in increased temporal metabolite stability due to reduced enzymatic activity.

**Methods:**

The metabolite content of six lysed non-heated (L-FB) and six lysed heated fibroblast samples (LH-FB) was measured as a function of time. Additionally, one sample each of non-heated and heated lysed adrenal cells (L-AD & LH-AD), which are less robust than fibroblasts, were measured. After lysis according to [1], half of the samples were heated (70°C) for 20min. <sup>1</sup>H-PROJECT [2] spectra were obtained on a Bruker Avance-II spectrometer (500.13MHz, 277K, MAS=3kHz) at different times over 9 hours. Individual peak analysis was performed investigating temporal changes. For fibroblasts, statistical methods were applied including PCA.

**Results and Discussion:**

PCA results and individual peak analysis clearly confirmed our hypothesis: The temporal metabolite stability for LH-FB was largely maintained in contrast to L-FB. Average temporal variation of all peaks was 14.4% for LH-FB compared to 43.1% for L-FB. Additionally, the PCA plot demonstrated close clustering for LH-FB, whereas L-FB were spreading out over time. Also LH-AD showed temporal metabolic stability, while L-AD exhibited strong changes. Our results suggest using cell lysis in combination with heating for extended long-term HR-MAS NMR measurements, in order to minimize metabolite content modifications over measurement time.

**References:**

1. Duarte IF, et al. Anal. Chem. 200981:5023.

2. Aguilar JA, et al. Chemical Communications 201248:811.



**Poster #: 191****Abstract #: 2151**

**Abstract Title:** Non-targeted metabolite profiling reveals metabolic alterations in dogs with behavioral abnormalities

**Authors:** Jenni Puurunen, Sini Sulkama, Katriina Tiira, Marko Lehtonen, Kati Hanhineva, Hannes Lohi,

**Presenting Author Affiliation:** University of Eastern Finland

**Abstract Submission:**

Behavioral abnormalities like fearfulness, noise phobia and attention deficit hyperactivity disorder (ADHD) are prevalent but poorly understood problems in dogs. Research, treatment and diagnostics of the corresponding human neuropsychiatric disorders is challenged by the complex etiology and clinical heterogeneity. Since inbred dogs naturally manifest neuropsychiatric traits that respond to human medication, they could offer genetically feasible and physiologically relevant animal model to understand molecular backgrounds of anxiety. As a part of our ongoing canine anxiety research program, this study aimed to identify fear- and ADHD-related candidate biomarkers by a metabolomics approach. We collected fresh plasma samples from diet-controlled 11 ADHD and 11 control German Shepherds and from 20 fearful and 21 non-fearful German Shepherds and Great Danes. Samples were analysed by liquid chromatography combined with mass spectrometry (LC-MS)-based non-targeted metabolite profiling. We found alterations in 18 metabolites in the ADHD dogs, including 3-indolepropionic acid, kynurenic acid and several phospholipids. Preliminary results show also fear-related changes, including increased glutamine. We found also breed-specific alterations in the metabolite profiles of fearful dogs. In summary, we demonstrate a promising metabolomics approach in canine anxiety and our findings in canine ADHD and fear agree with earlier findings in human and rodent models. However, larger replication studies are warranted to confirm and understand the biological roles of the identified metabolites in canine ADHD and fear.

**Poster #: 192**

**Abstract #: 2184**

**Abstract Title:** Phospholipid ratios in Zellweger Spectrum disorder patients

**Authors:** Katharina Herzog,

**Presenting Author Affiliation:** Academic Medical Center Amsterdam

**Abstract Submission:**

Peroxisomes are subcellular organelles involved in various metabolic processes, including fatty acid and phospholipid homeostasis. The Zellweger Spectrum disorders (ZSDs) represent a group of diseases caused by a defect in the biogenesis of peroxisomes. Accordingly, cells from ZSD patients are expected to have an altered composition of fatty acids and phospholipids.

Using an LC/MS-based lipidomics approach, we show that the phospholipid composition is characteristically altered in cultured primary skin fibroblasts from ZSD patients when compared to healthy controls. We observed a marked overall increase of phospholipid species containing very long chain fatty acids, and a decrease of phospholipid species with shorter fatty acid species in ZSD patient fibroblasts. In addition, we detected a distinct phosphatidylcholine profile in ZSD patients with a severe and mild phenotype when compared to control cells.

Based on our data, we present a set of specific phospholipid ratios for fibroblasts that clearly discriminate between mild and severe ZSD patients, and those from healthy controls. Our findings will aid in the diagnosis and prognosis of ZSD patients, including an increasing number of mild patients in whom hardly any abnormalities are observed in biochemical parameters commonly used for diagnosis.

**Poster #: 193**

**Abstract #: 2341**

**Abstract Title:** Application of a targeted metabolomics approach to investigate regulation of the biosynthetic pathway of melatonin in *Escherichia coli*

**Authors:** Mette Kristensen, Scott Harrison, Hao Luo, Konstantin Schneider, Rebecca Lennen, Markus Herrgard, Hanne Bjerre Christensen,

**Presenting Author Affiliation:** PhD

**Abstract Submission:**

Melatonin has been characterized as an important bioactive molecule and has been shown as a clinically effective drug e.g. exhibiting positive effects as a sleep aid. Commercial melatonin production is primarily accomplished by complex chemical synthesis and thus an alternative more sustainable production form is desirable. In this presentation, we demonstrate the analytical basis for microbial production of melatonin and its related intermediates.

We have developed an analytical method applying liquid chromatography coupled to a Fusion orbitrap mass spectrometry in order to accomplish a fast quantification of metabolites related to the melatonin pathway as well as the potential undesirable side products. The method take benefits of high resolution of the orbitrap mass spectrometer for compound identification and application of non-specific full scan experiments allows us to recognize potential involvement of non-desirable and yet competing pathways.

**Poster #: 194**

**Abstract #: 2038**

**Abstract Title:** The metabolic state in newborns and infants is linked to inflammasome activity and respiratory diseases

**Authors:** Ulrike Rolle-Kampczyk, Kirsten Offenberg, Mario Bauer, Wolfgang Otto, Stefan Röder, Konrad Grützmann, Ulrich Sack, Jan-Christoph Simon, Michael Borte, Martin von Bergen, Irina Lehmann, Gunda Herberth,

**Presenting Author Affiliation:** UFZ - Helmholtz Centre for Environmental Research

**Abstract Submission:**

**Background:** There is evidence that endogenous metabolites may regulate inflammatory processes. Here examine the impact of endogenous dietary metabolite concentrations on inflammasome activity at birth and in early childhood including the regulation by miRNAs.

**Methods:** Within our prospective birth cohort study LINA we performed a detailed metabolic profiling and analyzed the expression of inflammasome components (NLRP3, CASPASE-1), TLRs and the effector cytokines IL-1 $\beta$  and IL-18 in blood samples at birth (cord blood) and at children's age of one. Furthermore, the impact of these cytokines on disease outcomes was assessed. MicroRNA (miRNAs-223, -326, -155, 146a) expression was analyzed in cord blood samples.

**Results:** At birth as well as at the age of one hexose concentrations were positively whereas amino acids and lysophosphatidylcholines (lysoPCs) were negatively associated with TLR expression and the inflammasome components NLRP3 and CASPASE-1. Children with increased expression of NLRP3 and CASPASE-1 had higher blood levels of the effector cytokines IL-1 $\beta$  and IL-18. IL-1 $\beta$  concentrations were predictive for the development of bronchitis and wheezing in the first two years of life. NLRP3 and TLRs were positively associated with miRNAs-223, -326 and negatively associated with miRNA-155 and -146a, suggesting a regulatory role of these miRNAs.

**Conclusion:** At birth and in early infancy, inflammasome activity seems to be influenced by the balance between hexoses, amino acids and lysoPCs and may have an impact on the development of respiratory outcomes in early infancy.

**Poster #: 195**

**Abstract #: 2043**

**Abstract Title:** An untargeted metabolomics approach for to identify potential biomarkers in spontaneous pre-term birth (sp-PTB) delivery, using a label-free LC-DIA-MS approach

**Authors:** SHIRISH YAKKUNDI, Lee Gethings, Aude-Claire Morillon, James Langridge, Louise Kenny,

**Presenting Author Affiliation:** INFANT Centre, Cork University Maternity Hospital

**Abstract Submission:**

Pre-term delivery remains the leading cause of perinatal mortality and there is currently no clinically useful screening test. The BiomarkErs FOR Early Birth project is looking to exploit metabolomics for identification of a useful early pregnancy-screening test for sp-PTB. Birth before 37 weeks' gestation is the single biggest cause of neonatal deaths in the world.

Samples consisting of 15 and 20-week heparinised plasma from women whose pregnancies reached term gestations (Control, n=20) as compared to pregnancies subsequently complicated by sp-PTB prior to 34 weeks' gestation (Case, n=20) were used. Plasma was taken up for protein precipitation using methanol, followed by MTBE extraction to separate lipids from the polar metabolites. QC sample was constructed by pooling aliquots from all samples. Data were collected for all samples in triplicate using LC-MS on a hybrid quadrupole oa-TOF mass spectrometer (Synapt G2-S). The label-free data were normalised, processed and database searched using Progenesis QI (Nonlinear dynamics, UK).

The lipids and polar metabolite extracts were analysed on positive and negative ion mode on BEH and HILIC columns respectively. Samples were stratified, randomised and data was acquired, with the pooled QC sample injected every 10 injections. LC-MS data were acquired, using a data independent analysis (DIA) approach. Following peak picking and alignment, features were subsequently processed using multivariate statistical analysis (MVA). Supervised OPLS discriminate analysis was used to filter for features of significant correlation and covariance prior to identification. Data (combined from positive and negative) were searched against the Human Metabolite Database (HMDB), ChEBI and LipidMaps. Identifications were scored on the basis of mass accuracy, isotopic fit and fragmentation. Data were further filtered to only include identifications fulfilling mass accuracy <5 ppm,  $p < 0.05$  and CV <30%. Initial identifications provided a variety of metabolite classes including long chain fatty acids, ceramides, sphingomyelins (SM) and organic acids.

**Poster #: 196**

**Abstract #: 2094**

**Abstract Title:** Simultaneous standardized quantification of metabolites and proteins in tear fluid

**Authors:** Sascha Dammeier, Janina D'Alvise, Dario Bosch, Michael Seid, Franziska Klose, Spyridon Dimopoulos, Marius Ueffing, Focke Ziemssen,

**Presenting Author Affiliation:** Eberhard Karls University Tuebingen

**Abstract Submission:**

Tear fluid represents an easily accessible body fluid that has the potential to become a matrix for diagnostic analyses both for eye-related diseases and systemic disorders. In this context, it has been shown that measuring the glucose concentration in tear fluid can serve as a surrogate for the invasive determination of the blood glucose level, e.g. in case of monitoring diabetes. Moreover, as tear fluid has multiple functions like protection of epithelial cells against dehydration, carrying nutrients, and prevention of microbial invasion its composition is reflective of ocular health. To elucidate the homeostasis of tear fluid by investigating the major metabolite components and proteins we sought to establish a standardized protocol for combined targeted metabolomics and targeted proteomics. Therefore special attention was turned to the process of sample taking, to the parallelization of proteomics and metabolomics, and to the analysis of intra- and inter-individual variation. Tear fluid was taken from volunteers by special Schirmer strips at least on three different days. For further processing 4 mm punches of the strips were used. We prepared a metabolite fraction by using a commercial kit leaving the precipitated proteins behind. The remaining material was processed for bottom-up proteomics. Targeted metabolite analysis could be performed for more than 100 analytes, i.e. amino acids, acylcarnitines, glycerophospholipids, etc. Most of the major metabolite quantifications exhibited good intra-individual reproducibility with CV < 20 %. For targeted proteomics we established an assay based on selected-ion monitoring for ten abundant tear proteins like lactotransferrin, lipocalin-1, hemopexin etc. using pre-selected proteotypic peptides with high reproducibility. Since substantial variances in individual tear secretion rates represent a major issue we will present the evaluation of different strategies to normalize the data. The achieved level of standardization raises hope for future diagnostic applications using tear fluid, e.g. by multiplexing biomarkers.

**Poster #: 197**

**Abstract #: 2108**

**Abstract Title:** Improving a biosignature for respiratory chain deficiencies

**Authors:** Roan Louw,

**Presenting Author Affiliation:** North-West University

**Abstract Submission:**

Mitochondria are the main energy producing site in the cell and found in nearly all eukaryotic cells. These organelles generate cellular energy in the form of adenosine triphosphate (ATP), mostly by means of the oxidative phosphorylation system, consisting of the respiratory chain (RC) and ATP synthase complex. A respiratory chain deficiency (RCD) results when one of the four complexes of the RC becomes impaired. Diagnosing RCDs is a major challenge and includes clinical, histochemical, molecular and biochemical assessment, where the golden standard remains enzyme analyses on muscle sample, a highly invasive procedure. Recently a urinary RCD biosignature was proposed, with the potential to be used as a screening tool for selecting patients to undergo a muscle biopsy for the possible diagnosis of a RCD. The proposed biosignature however consists of 12 LC-MS features that have not been verified. Thus the aim of this study was to improve this proposed biosignature, by verifying the 12 features and expanding the biosignature using additional analytical platforms.

Verification of the LC-MS features was performed on different analytical platforms and software. Five of the 12 features could be verified and only one could be identified to a certain extent. Gas-chromatography mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) analysis was used to expand the biosignature. Data mining steps and statistical analyses were performed to compile a list of top ranked features for each platform. From this a final list (biosignature) of five features was compiled and the discriminative power was evaluated. Results indicated that the improved biosignature was unable to classify samples 100% accurately with some of the clinical control samples classified as RCDs. However the biosignature could still be helpful in limiting the inclusion of clinical control patients in the biopsy process.

**Poster #: 198**

**Abstract #: 2264**

**Abstract Title:** Mitochondrial DNA Replication Defects Disturb Cellular dNTP Pools and Remodel One-Carbon Metabolism

**Authors:** Vidya Velagapudi,

**Presenting Author Affiliation:** Institute for Molecular Medicine Finland FIMM

**Abstract Submission:**

Mitochondrial dysfunction affects cellular energy metabolism, but less is known about the consequences for cytoplasmic biosynthetic reactions. We report that mtDNA replication disorders caused by TWINKLE mutations—mitochondrial myopathy (MM) and infantile onset spinocerebellar ataxia (IOSCA)—remodel cellular dNTP pools in mice. MM muscle shows tissue-specific induction of the mitochondrial folate cycle, purine metabolism, and imbalanced and increased dNTP pools, consistent with progressive mtDNA mutagenesis. IOSCA-TWINKLE is predicted to hydrolyze dNTPs, consistent with low dNTP pools and mtDNA depletion in the disease. MM muscle also modifies the cytoplasmic one-carbon cycle, transsulfuration, and methylation, as well as increases glucose uptake and its utilization for de novo serine and glutathione biosynthesis. Our evidence indicates that the mitochondrial replication machinery communicates with cytoplasmic dNTP pools and that upregulation of glutathione synthesis through glucose-driven de novo serine biosynthesis contributes to the metabolic stress response. These results are important for disorders with primary or secondary mtDNA instability and offer targets for metabolic therapy.

**Citation:**

Nikkanen, J., Forsström, S., Euro, L., Paetau, I., Kohnz, R.A., Wang, L., Chilov, D., Viinamäki, J., Roivainen, A., Marjamäki, P., Liljenbäck, H., Ahola, S., Buzkova, J., Terzioglu, M., Khan, N.A., Pirnes-Karhu, S., Paetau, A., Lönnqvist, T., Sajantila, A., Isohanni, P., Tynismaa, H., Nomura, D.K., Battersby, B., Velagapudi, V., Carroll, C.J\*, Suomalainen, A\*. Mitochondrial DNA Replication Defects Disturb Cellular dNTP Pools And Remodel One-Carbon Metabolism. *Cell Metabolism*. 2016 S1550-4131(16)30012-2. (Epub ahead of print). PMID: 26924217.



**Poster #: 199**

**Abstract #: 2333**

**Abstract Title:** Identification of Phospholipid Species Implicated in Dementia by Untargeted High Resolution LC/MS and Data Dependent MS/MS

**Authors:** David Peake, Mandy Bowman, Reiko Kiyonami,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

Alzheimer's disease (AD) is the leading cause of dementia in the elderly. Recently, Mapstone et al. (Nature Medicine 201420(4):415-418) reported plasma phospholipids that predicted cognitively normal adults who later progressed to either mild cognitive impairment or dementia due to AD. This study used a targeted metabolomics p180 kit (Biocrates, Life Science AG, Austria) that measures phospholipids by infusion and selected reaction monitoring (SRM). It is not possible to assign the phospholipid species using this low-resolution SRM approach. Identification of the fatty acid constituents of the phospholipids implicated in AD is critical to determine their function and contribution to pathophysiology of the disease and to develop quantitative assays.

The objective of this study is to identify 7 phosphatidylcholine (PC) lipids in plasma. Pooled plasma from 15 participants with normal cognition (n=5), mild cognitive impairment (n=5), and dementia (n=5) was extracted using chloroform, methanol and water containing LPC 17:1 and PC 17:0/14:1 (Avanti Polar Lipids, Alabama). Plasma lipids were separated by ultrahigh-pressure liquid chromatography (Dionex 3000-RSLC) using a C30 Acclaim column (2.1 X 150 mm, 3.0  $\mu$ m) and analyzed with a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer. LC/MS and MS/MS data were obtained at 120,000 and 30,000 mass resolving power, respectively in positive and negative mode.

Thermo Scientific LipidSearch 4.1 software was used for molecular lipid identification. Plasma PC 36:6 is primarily 14:0\_22:6 PC 40:2 is 22:0\_18:2 and PC 40:6 is 18:0\_22:6. PC 40:6e is a mixture of 18:1e\_22:5 and 20:2e\_20:4 and PC 38:6 is a mixture of 16:0\_22:6, 18:1\_20:5 and 18:2\_20:4 species. However, the fatty acid molecular identity for PC 38:0 and PC 40:1 were not confirmed. PC 18.1e\_22:6 was identified instead of PC 38:0 by negative ion MS/MS. These phospholipid fatty acid compositions determined here enable development of quantitative methods in order to understand their potential roles in AD.

**Poster #: 200**

**Abstract #: 2449**

**Abstract Title:** Innovations and Applications of Quantitative Metabolomics in Human Health

**Authors:** Rupasri Mandal, Lu Deng, Avalyn Stanislaus, Rosa Vázquez-Fresno, Reza Rezaei Darestani, Yiran Zhang, Beomsoo Han, Trent Bjorndahl, Ying (Edison) Dong, AnChi Guo, Bob Stanley, Danuta Chamot, James Harynuk, Liang Li, Christoph Bo

**Presenting Author Affiliation:** The Metabolomics Innovation Centre

**Abstract Submission:**

TMIC (The Metabolomics Innovation Centre) is Canada's national metabolomics laboratory. It specializes in performing quantitative metabolomics assays on human, animal, plant and microbial samples. TMIC uses a wide range of quantitative metabolomic technologies based on NMR, GC-MS, GCxGC-TOF, LC-MS/MS, LC-FT/MS, HPLC-UV/FD, ICP-MS and HPLC-ELSD-FAMES-MS. In order to keep pace with the rapid developments in metabolomics, 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, oxylipins, one-carbon metabolites, organic acids, volatiles and steroids. TMIC has also completed the development of a series of quantitative metabolomics kits (NMR, GC-MS and LC-MS-based) consisting of reagents, protocols and/or software that can simultaneously, inexpensively and quantitatively measure a large number of metabolites in human biofluids. These kits are designed to be compatible with most commonly available analytical platforms. TMIC is also involved in the systematic characterization of human biofluids. Using multi-platform, quantitative metabolomic techniques along with extensive literature surveys, TMIC completed the measurement of 4229 metabolites in the human serum metabolome, 419 metabolites in the human CSF metabolome, 2882 metabolites in the human urine metabolome and 853 metabolites in the human saliva metabolome. Together, these efforts represent the most comprehensive metabolomic characterizations of any human biofluid achieved to date. TMIC has also used quantitative metabolomic techniques that have led to the identification of predictive biomarkers for early-/ late-onset preeclampsia, Down syndrome, fetal congenital heart defects, colonic polyps, heart failure and pediatric kidney transplant rejection. Some of these biomarker panels are now being moved into clinical practice.

**Poster #: 201**

**Abstract #: 2455**

**Abstract Title:** Routine plasma preparation protocols and their influence on the metabolome

**Authors:** Dorothea Lesche, Roland Geyer, Daniel Lienhard, Christos Nakas, Gaëlle Diserens, Peter Vermathen, Alexander Leichtle,

**Presenting Author Affiliation:** University Institute of Clinical Chemistry

**Abstract Submission:**

Blood plasma is a widely employed sample matrix for metabolomics. It is usually generated by centrifuging whole blood with defined RCF (relative centrifugal force „g“), time, and temperature, followed by transferring the supernatant in a new vial to permanently separate liquid and cellular blood components. Whereas plasma preparation standard operating procedures (SOPs) are generally strictly followed within labs, they might substantially vary between them. In our study, we investigated the differences in differentials, NMR- and UPLC-qTOF profiles introduced by different routinely used plasma preparation procedures.

For our study, we collected two identical whole blood samples from each participant, measured whole blood differentials, centrifuged the samples separately according to two different plasma preparation protocols (clinical chemistry [3000g 5' ...] vs. hemostaseology [1500g 10' ...]) currently in use at our hospital, and generated three aliquots of each sample (60 in total). We measured plasma differentials (Sysmex XN 9000), NMR profiles (Bruker Avance II 500MHz) and UPLC-qTOF profiles (Waters Synapt G2-S) and compared the protocol-based differences by paired Wilcoxon signed rank test and multilevel sparse partial least squares discriminant analyses (multilevel sPLS-DA), respectively.

For both protocols, we found significant differences in platelet count and, based on distinct metabolites, perfect separation of the samples of both protocols in NMR- as well as UPLC-qTOF sPLS-DA analyses. While in-depth data analysis is still ongoing, several analytes of the amino acid and lipid metabolism could be identified as discriminating features.

Our results demonstrate that different routinely used plasma preparation protocols can yield distinct metabolome patterns on NMR- and TOF-based platforms. This may bias metabolomics studies incorporating samples from various centers, and emphasizes the need for standardization of plasma preparation procedures.

**Poster #: 202**

**Abstract #: 2120**

**Abstract Title:** Opportunity looms: metabolic regulation of an opportunistic pathogen

**Authors:** Volker Behrends,

**Presenting Author Affiliation:** University of Roehampton

**Abstract Submission:**

The Gram negative bacterium *Pseudomonas aeruginosa* is a prime example of an opportunistic bacterium as it infects hosts including plants, invertebrates and mammals. In humans, it is particularly important in the context of hospital-acquired infections as well as chronic infections of the lungs of cystic fibrosis patients.

We were interested in the interplay between modes of 'attack' and 'defense' in the organism, in particular how regulation of metabolite uptake is linked to stress resistance and production of virulence factors with cross-kingdom efficacy like cyanide and pyocyanin. To assay metabolic regulation, we used NMR spectroscopy and mass spectrometry-based methods on *P. aeruginosa* wild types and mutants in key regulatory systems.

We found that while metabolic uptake is redundantly regulated, elimination of elements of the catabolite repression system led to loss of efficiency in metabolism, complex compensatory behaviour affecting virulence and a reduction in stress tolerance and antibiotic resistance. This is in line with previous findings that link metabolic alterations in *P. aeruginosa* to a loss in one or more stress resistance pathways.

We propose that knowledge of metabolism could open new avenues for using metabolite-based therapies to combat the growing problem of antibiotic resistance in *P. aeruginosa* and other bacterial species.

**Poster #: 203**

**Abstract #: 2067**

**Abstract Title:** METABOLIC EFFECTS IN ATORVASTATIN-TREATED LIVER CELLS

**Authors:** Gabriela Venturini, Kallyandra Padilha, Pamella Malagrino, Tamiris Gois, José Krieger, Alexandre da Costa Pereira,

**Presenting Author Affiliation:** Heart Institute of University of Sao Paulo

**Abstract Submission:**

Statins are widely used for treatment of heart and cardiovascular diseases due effective cholesterol-lowering 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase inhibitors. Others pleiotropic effects are also known, as improving endothelial function, inhibiting vascular inflammation and oxidation, and increase of many lipid-related protein including HMGCR, FDFT, SQLE, and LDLR. This study aimed to analyze metabolomic profiling from liver carcinoma cell line (HepG2) treated with atorvastatin by MS-based metabolomics. In this work, HepG2 was plated in 55cm<sup>2</sup> dishes (1x10<sup>7</sup> cells/dish) and cultured with DMEM/high. Cells were treated with 15µM atorvastatin and after 24hours of treatment, they were harvest with acetonitrile/isopropanol/water solution (3:3:2) to metabolite extraction, followed by lyophilization. To derivatization, the extract was spiked with 50uL methoxyamine in pyridine solution, followed by trimethylsilylation adding MSTFA/TMCS. For each samples was add 5µL of internal standard solution (d27-myristic acid) and 5 µL of FAME. 1µL of this derivative was used for GC/MS analysis (Agilent 5977A Series GC/MSD). Analysis was performed with NIST 11 and FiehnA.01.00 compound library using Unknowns - Agilent MassHunter Workstation Quantitative Analysis. Data were analyzed in Metaboalyst. By GC-MS technique and statistical analysis were possible to identify 31 metabolites differentially changed in cells treated and non-treated with atorvastatin. According to system biology analysis, these changed metabolites are related to cellular growth and proliferation, cellular assembly and organization, cellular development, cell cycle, cell death and survival. We identified L-threonine, tagatose, L- sorbose, O-phosphocolamine, aspartic acid and L-glutamic acid as upregulated in atorvastatin-treated cells. On the other hand, citric acid, adenosine, D-malic acid, adenosine-5-monophosphate, cytidine-5'-monophosphate, DL-3-aminoisobutyric acid, N-acetyl-L-aspartic acid and glycerol 1-phosphate were found as downregulated in the same condition. Our data suggest changed mainly in fatty acids and carboxylic acids metabolism. These metabolites could be useful to better understand the metabolism effects of the drug in the patients who are submitted to atorvastatin treatment.

**Poster #: 204**

**Abstract #: 2079**

**Abstract Title:** Sampling Methods for Profiling Plant Volatiles Profiling by GCxGC-TOFMS

**Authors:** James Harynuk, A. de la Mata, Chaminda Weeraddana, Maya Evenden, Lawrence Adutwum,

**Presenting Author Affiliation:** University of Alberta

**Abstract Submission:**

When plants are infected by pathogens, their volatile organic compound (VOC) profile may be altered. This may, in turn, alter the responses of foraging herbivores in the system. In the canola agroecosystem of the Canadian Prairies, club root disease, caused by a soil borne obligate biotrophic protist, *Plasmodiophora brassicae*, causes large reductions in yield. Infection may also predispose canola plants to attack by other pests or

prime a defensive response that could protect the plant from subsequent attack. Female moths of the generalist herbivore, the Bertha armyworm, *Mamestra configurata*, can distinguish between infected and uninfected canola plants and prefer to lay eggs on uninfected plants. Clubroot resistant-canola varieties however, when infected with the pathogen are more attractive to female Bertha armyworm moths than uninfected plants of the same variety. To probe changes in volatile signatures from various canola plants, passive and active sampling strategies have been investigated. Passive sampling was performed by activated carbon strips, commonly used in passive headspace sampling of fire debris, and active sampling with sorbent tubes containing various sorbents were performed. After collection, volatiles were desorbed and analyzed by GCxGC-TOFMS.

**Poster #: 205**

**Abstract #: 2158**

**Abstract Title:** NMR-based metabolic profiling of field-grown leaves from sugar beet plants harbouring different resistance levels to *Cercospora* leaf spot disease

**Authors:** Yasuyo Sekiyama, Kazuyuki Okazaki, Seishi Ikeda, Jun Kikuchi,

**Presenting Author Affiliation:** Food Research Institute, NARO

**Abstract Submission:**

*Cercospora* leaf spot (CLS), caused by the fungus *Cercospora beticola* Sacc., is one of the most serious leaf diseases of sugar beet (*Beta vulgaris* L.) plants worldwide. The breeding of sugar beet varieties with high-level CLS resistance and high yields is a major challenge for breeders. No efficient technique for the screening of sugar beet plants for CLS resistance has been proposed, as environmental factors largely affect the expression of resistance under field conditions. An environmental metabolomic approach could be useful to shed light on the underlying mechanism of CLS resistance in relation to various environmental factors in agricultural fields. This approach may also facilitate the assessment of CLS resistance levels in a manner complementary to DNA marker-assisted breeding. Here, we report on the NMR-based metabolic profiling of field-grown leaves from sugar beet genotypes harbouring different CLS resistance levels.

Leaves were collected from 12 genotypes of sugar beet grown in 2015 (Hokkaido, Japan) at three time points: (i) in the middle of May, at the seedling stage, just prior to planting in an experimental field (41 days after seeding) (ii) in the middle of June, during the early growth stage (37 days after field planting) and (iii) in the middle of July, during the root enlargement stage (61 days after field planting and 14 days after the inoculation of *C. beticola*-infected old leaves [1]). <sup>1</sup>H NMR spectra of foliar metabolites soluble in a D<sub>2</sub>O-based buffer were measured and subjected to a multivariate analysis. Principal component analysis showed class separation corresponding to the growth stage of the plants. In addition, the differences in metabolite profiles between resistant and susceptible cultivars varied according to the growth stage. Details of the metabolic changes at each time point will be presented.

[1] Taguchi et al., *G3-Genes Genom Genet*, 1, 283–291 (2011).

**Poster #: 206**

**Abstract #: 2281**

**Abstract Title:** Effect of the rootstock-scion grafting combination on stress tolerance and fruit quality of mandarin (*Citrus reticulata*)

**Authors:** Snehil Srivastava,

**Presenting Author Affiliation:** Ben-Gurion University

**Abstract Submission:**

Grafting offers the advantage of implementing a variety of scion/rootstock combinations for a particular species or cultivar with the aim to improve properties like plant vigor and stress tolerance. To investigate whether grafting can yield fruits with improved quality and/or better tolerance to stress, we studied three different varieties of *Citrus reticulata* (mandarin), 'Orra Shani', 'Meirav' and 'Michal', grafted onto three different rootstocks, sour orange [*C. aurantium* (L.)], Volkameriana lemon [*C. volkameriana* (Ten. and Parq)] and US-812 [*C. sunki* (Hort. ex Tan.) × *Poncirus trifoliata* (L.)]. After 10 years of tree growth, metabolic profiling of fruit juice was conducted using gas chromatography-mass spectrometry (GC-MS). Our results showed that fruit juice from 'Meirav', vs 'Orra Shani' and 'Michal' has higher levels of amino acids, like asparagine and alanine, that are components of the aroma volatiles that contribute to fruit quality. Moreover, 'Orra Shani' shows relatively high level of stress related metabolites like 4-aminobutanoic acid (GABA) and pyroglutamic acid irrespective of its three rootstocks (sour orange, Volkameriana and US-812). Principal component analysis (PCA) of mandarin juice indicated that the rootstock has a limited or negligible effect on fruit quality. To validate this hypothesis, GC-MS analysis of phloem sap from the scion and rootstock of all nine combinations is underway. Nitrogen assimilation analysis [inductively coupled plasma–atomic emission spectrometry (ICP-AES)] of both phloem sap and fruit juice is also underway with the aim to elucidate the translocation of specific elements, like nitrogen, phosphorus and potassium, in all nine scion-rootstock combinations. In addition, secondary metabolite analysis using headspace GC-MS will be performed to reveal their roles in metabolic pathways that may affect the quality of mandarin fruits.



**Poster #: 207**

**Abstract #: 2148**

**Abstract Title:** Varietal characteristics of apple aroma associated with sensory attributes

**Authors:** Fukuyo Tanaka, Fumiyo Hayakawa, Miho Tatsuki, Keiki Okazaki, Yukari Kazami, Satoshi Kasai,

**Presenting Author Affiliation:** NARO Agricultural Research Center

**Abstract Submission:**

The characteristics of five apple cultivars developed in Japan were analyzed by sensory and instrumental analysis with the aim of investigating the relationship between fruit characteristics (texture, taste, and odor), flavor profiles, and ethylene emission.

**Samples.** The following cultivars were used: Fuji (the most popular and standard cultivar in Japan), Romu 50 (Kaori) and Aori 21 (Shunmei 21<sup>®</sup>), which are elastic in texture and frequently watercored, Aori 15 (Hoshi no Kinka<sup>®</sup>) and Ohrin, which are softer and richer in aroma. They were stored at 0°C under atmospheric composition and analyzed about 10, 30, and 100 days after harvest (DAH).

**Analysis.** A quantitative descriptive analysis (QDA) of flavors and textures was performed by seven trained panelists. The profiling of 69 volatile compounds was performed by headspace trap using the MonoTrap<sup>TM</sup> (a silica monolith for trapping the compounds of interest) and a thermal desorption (TD)/GC/MS system.

**Results and discussion.**

**Volatile compounds.** At 10 DAH, most volatile compounds were low in all cultivars, except in Hoshi no Kinka<sup>®</sup>. This cultivar was abundant in propyl, butyl, and hexyl esters of acetic acid. At 30 DAH, volatile compounds, such as butyl and hexyl esters of butanoic and hexanoic acids, increased in Ohrin, a high ethylene-producing cultivar. The cultivars Kaori, Shunmei 21<sup>®</sup>, and Fuji, which are low ethylene-producing cultivars, developed watercore and produced various ethyl esters with fruity, floral, and sweet odors.

**QDA.** At 10 DAH, most cultivars had a perceived green and sour odor and a crisp elastic texture. The sensory attributes of the five cultivars were most distinctive at 30 DAH, which changed to sweet, banana-like and ripe, fruit-like odors and soft texture during the following two months, except in Shunmei 21<sup>®</sup>. The relationship between each volatile compound, sensory perception, and quality properties, such as Brix, acidity, and hardness, is also discussed.

**Poster #: 208**

**Abstract #: 2210**

**Abstract Title:** The effects of genetic background and cultivation methods on the phytochemical profile of strawberries

**Authors:** Anna Kårlund, Marko Lehtonen, Reijo Karjalainen, Kati Hanhineva,

**Presenting Author Affiliation:** University of Eastern Finland

**Abstract Submission:**

Strawberry is a nutrient-dense fruit that is globally highly appreciated. In addition, leaves of strawberry have evoked interest as a promising raw-material for health-related applications. Environment and genotype are important determinants of strawberry phytochemical content, and the ability of strawberry cultivars to maintain their chemical composition in different cultivation conditions varies. Wide-scale metabolite profiling can be utilized to further analyze the large phytochemical repertoire of both fruits and leaves of strawberry to investigate the impact of cultivation conditions as well as genetic background on chemical composition and content.

We surveyed the metabolite profiles of conventional (15 cultivars) and organic (3 cultivars) strawberries and strawberry leaves (6 cultivars) using non-targeted metabolite profiling with LC-qTOF-ESI-MS. Major molecular features were tentatively annotated on the basis of the fragmentation patterns in the data-dependent MS/MS acquisition. Cluster analyses and principal component analysis were utilized to clarify the metabolic similarities and differences between strawberry cultivars and between organic and conventional strawberry fruits.

Specific classes of flavonoids, tannins and phenolic acids are the most characteristic phenolic compounds in strawberries, but demonstrate significant, cultivar-dependent differences in abundance. Terpenoids and long-chain fatty acids, e.g. linolenic and other octadecatrienoic acids are on high levels in both strawberry fruits and leaves. Interestingly, a sapogenin compound was found to be on higher level in organically grown strawberry fruits in comparison to conventional fruits. Furthermore, the levels of versatile, glycosidically bound aroma precursors fluctuate between cultivars, and along with phenolics and lipids, are suggested to contribute to the cultivar-specific horticultural and quality attributes. The compositional differences between cultivars were observed to be much more pronounced than the differences caused by cultivation methods. The results indicate that non-targeted metabolite profiling is an effective tool to evaluate the composition and stability of phytochemicals in strawberry cultivars, both fruits and leaves, grown in different environmental conditions.

**Poster #: 209**

**Abstract #: 2024**

**Abstract Title:** LC-MS METABOLOMICS SHOWS A SMART WAY TO REDUCE SULFITES IN WINE

**Authors:** Panagiotis Arapitsas, Maurizio Ugliano, Paolo Pangrazzi, Daniele Perenzoni, Andrea Angeli, Fulvio Mattivi,

**Presenting Author Affiliation:**

**Abstract Submission:**

What does happen in wine in the presence of oxygen? What is the fate of exogenous antioxidants such as SO<sub>2</sub>? A consortium between a winery, a wine stopper producer and a MS metabolomics laboratory, was build to answer the above questions towards an ambitious project. The experimental design included 216 bottles of 12 different white wines produced from 6 different cultivars (Inzolia, Muller Thurgau, Chardonnay, Grillo, Traminer and Pinot gris). Half of them were bottled using the standard industrial process with inert headspace and the other half without inert gas and with extra headspace. After 60 days of storage at room temperature, the wines were analysed using an untargeted LC-MS method [1].

The use of a detailed metabolomics workflow, with several levels of quality control and marker selection, gave 35 metabolites putatively induced by the different amounts of oxygen. These metabolite markers included ascorbic acid, tartaric acid and various sulfonated compounds observed in wine for the first time (e.g. S-sulfonated cysteine, S-sulfonated glutathione and S-sulfonated pantetheine, sulfonated indole-3-lactic acid hexoside and sulfonated tryptophol). The consumption of SO<sub>2</sub> mediated by these sulfonation reactions was promoted by the presence of higher levels of oxygen on bottling [1].

The reaction between SO<sub>2</sub> and other antioxidants present in wine, like glutathione, results in depleting each other concentration [1]. So instead to have a synergic or additive protection due to the presence of multiple antioxidants, the wine is less protected from oxidations because of the antagonism between the antioxidants. This phenomenon, unknown until today, was pushing often winemakers to increase the added dose of SO<sub>2</sub> without knowing why, and as result to increase sulfites concentration in wine.

**Reference**

1. P. Arapitsas, M. Ugliano, D. Perenzoni, A. Angeli, P. Pangrazzi, F. Mattivi J. Chrom. A, 1429, 155-165 (2016)

**Poster #: 210**

**Abstract #: 2214**

**Abstract Title:** UNTARGETED METABOLOMIC STUDY FOR IDENTIFICATION OF BIOACTIVE METABOLITES AS POTENTIAL HEALTH PROMOTING AGENTS IN GRAPE TISSUES

**Authors:** Laura Millan, Alicia Sanchez, Ramon Barrio, Maria Aranzazu Goicolea, Carmen Sampedro,

**Presenting Author Affiliation:** University of the Basque Country (UPV/EHU)

**Abstract Submission:**

Plant-based foods are situated at the base of the food pyramid and some of them, such as fruits, vegetables and wine, contain thousands of phytochemicals that exert biological activities. Both food quality and its effect on human health have become a fundamental issue all over the world.

As far as wine science is concerned, metabolomics has been indispensable so as to extend the knowledge about the chemical composition of the grape. Both grape and wine are considered complex matrices made up of thousand of constituents. In particular, the chemical diversity of grapevine (*Vitis vinifera* L.) is mostly affected by secondary metabolites (alkaloids, terpenes, volatile oils, tannins, sterols, saponins and phenolics), among which phenolics is the most studied family due to their biological activity.

The goal of this research work seeks to investigate the metabolic profile of grape tissues (pulp, peel and seeds), focusing on non-polar components, comparing different grape varieties from Rioja region (Tempranillo, Graciano, Cabernet Sauvignon, Garnacha, White Garnacha and Viura), with the aim of finding significant clustering. Moreover, the resulting significant entities in class predictions tried to be identified as potential bioactive metabolites for improving human health.

Optimum method characteristics are essential in order to cover a broad range of chemical entities, starting with a minimal sample preparation, specially preferred for untargeted applications. Reverse phase liquid chromatography (RPLC) under gradient elution program is by far the mostly used separation mode for metabolome characterization and analysis. The LC system was combined with two API interfaces (APCI/ESI) to the accurate mass QTOF mass analyzer, covering less polar to non-polar entities. Data processing and statistical analysis are key steps in the metabolic workflow. Spectral dataset was reduced to a small number of principal components before class prediction analysis.

**Poster #: 211****Abstract #: 2315****Abstract Title:** A METABOLOMICS APPROACH TO CHARACTERIZE RAW, PASTEURIZED AND ULTRA-HIGH TEMPERATURE MILK USING UPLC-QTOF-MS and MULTIVARIATE DATA ANALYSIS**Authors:** Zhang Yangdong, Adam Ladak, Nan Zheng, zhengwei Jia, Xiaojie Tan, Fang Wen, Songli Li, Shengguo Zhao, Jiaqi Wang, Narendra Meruva, Jennifer Burgess,**Presenting Author Affiliation:** Ministry of Agriculture Laboratory of Quality & Sa**Abstract Submission:**

Bovine milk is heat treated to pasteurized (185.0°F, 15 s) and UHT (278.6°F, 15 s) milk prior to human consumption and before it is processed into a variety of dairy products. In this study, UPLC-QTOF-MS integrated with multivariate data analysis was applied to investigate the impact of heat treatment on milk composition and its nutritional quality. Milk metabolomics poses significant analytical challenges as bovine milk is a complex mixture of proteins, lipids, vitamins, carbohydrates and metal salts. The high resolution mass spectrometry (HRMS) based metabolite profiling workflow used in this study provides identification and quantification of wide range of milk metabolites.

A combination of UPLC separation, QTOF-MS detection and informatics was used to identify differences in milk samples. Three different milk samples (fresh, pasteurized and UHT milk, 10 replicates) and a pooled composite sample (1:1:1 ratio) were investigated. Samples from different groups were centrifuged to remove the top fat layer and the proteins in skimmed milk were precipitated. The supernatant was analyzed in a random order using UPLC-QTOF-MS in electrospray positive and negative modes.

Following UPLC separation and QTOF-MS detection, the raw data files were processed using Progenesis QI. Following peak alignment, detection and deconvolution, more than 34,000 compound ions in electrospray positive mode and 15,000 compound ions in electrospray negative mode were detected. Compounds that show significant differences between groups were short-listed using various multivariate analysis (MVA) techniques from principle component analysis (PCA), discriminant analysis (OPLS-DA) and correlation analysis.

Multiple structural assignment tools available within Progenesis QI such as accurate mass, retention time, isotopic distribution, theoretical fragmentation, measured fragmentation, elemental composition and searchable databases were utilized to improve confidence in compound identification. A customized milk metabolites database which included amino acids, vitamins and carbohydrates was used for identification.

**Poster #: 212****Abstract #: 2199****Abstract Title:** Metabolomics analysis of liver to reveal profiles disruption in bovines upon steroid treatment**Authors:** Giancarlo Biancotto, Roberto Stella, Eleonora Mastrorilli, Roberto Angeletti, Davide Bovo, Cristina Bellesso, Gaud Dervilly-Pinel, Anne-Lise Royer, Bruno Le Bizec,**Presenting Author Affiliation:** Istituto Zooprofilattico Sperimentale delle Venezi**Abstract Submission:****ABSTRACT**

The surveillance of illegal anabolic practices in bovine meat production is necessary to guarantee consumers' health. Screening strategies based on the recognition of indirect biological effects are considered by the community as promising tools to overcome some limitations of classical analytical methods and might therefore concur to ensure safer food for the consumer.

Given that hormonal therapies influence the physiology of an organism, strategies based on the detection of metabolic changes that occur following anabolic practices are promising approaches to identify their misuse [1-3]. The present work is aimed at characterizing the metabolic profile induced in liver by administration of anabolic steroids, and to identify potential disturbances in the hepatic metabolism. A total of 32 liver samples, 16 from untreated bulls and 16 from bulls treated with an ear implant (Revalor-XS®) containing trenbolone acetate (200 mg) and estradiol (40 mg), were analyzed following a LC-HRMS-based metabolomics analysis using RP and HILIC chromatographic separations. Different multivariate statistical tools were applied to the datasets to select common metabolites for classification of samples and to reveal potential biomarkers on the basis of their significant changes in concentrations after administration of sexual steroids. Currently, the identity of 8 candidate biomarkers is undergoing a confirmatory analytical process. Moreover, a subset of biomarkers was also validated by a different laboratory that performed the same analyses using an independent instrumental and elaboration platform, confirming the robustness of the results achieved.

**References**

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**Poster #: 213**

**Abstract #: 2208**

**Abstract Title:** Correlation with microbial diversity and MS-based metabolite profiling of doenjang, a fermented soybean paste during industrial process

**Authors:** Sunmin Lee, Sarah Lee, Ji Young Oh, Eun Jung Jeon, Dong Wan Lee, Beom Seok Kim, Choong Hwan Lee,

**Presenting Author Affiliation:** Konkuk university

**Abstract Submission:**

Doenjang with two different processes, industrial process (IP) and modified industrial process (mIP) by inoculating specific microorganisms, were subjected to metabolite profiling by liquid chromatography chromatography (LC-MS) and gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) to identify different metabolite in process and gain a better understanding of metabolism on diverse microbial population. In partial least squares discriminant analysis of primary and secondary metabolites, the patterns were clearly distinguishable at various processing steps (step 1: steaming, step 2: drying, step 3: meju fermentation, step 4: freezing, step 5: brining, step 6: doenjang aging) both IP and mIP. Most of the monosaccharides, amino acids, and fatty acids increased in steps 3-6. Isoflavones were major secondary metabolites identified during the processing of doenjang. Isoflavone glycosides, except malonyl glucoside, increased until step 3 and gradually decreased after step 3, while isoflavone aglycones showed the opposite tendency. The bacterial community analysis based on denaturing gradient gel electrophoresis showed bacteria and fungi contributed each processing steps. Correlation analysis microorganisms and metabolites indicated that *Aspergillus* population was correlated with the metabolism of the sugars, *Bacillus* with fatty acids, *Tetragenococcus* and *Zygosaccharomyces* with amino acids for doenjang fermentation. These results suggest that doenjang component and quality were strongly correlated with the existed microorganisms through correlation with MS-based metabolite profiling and microbial diversity during industrial process.

**Poster #: 214**

**Abstract #: 2420**

**Abstract Title:** <sup>1</sup>H NMR-based metabolomic analysis for diagnosis of uncomplicated urinary tract infection (UTI)

**Authors:** Milena Lussu, Tania Camboni, Francesco Del Carratore, Corrado Serra, Aldo Manzin, Luigi Atzori,

**Presenting Author Affiliation:** University of Cagliari

**Abstract Submission:**

Urinary tract infection (UTI) is one of the most common diagnosis in girls and women, and at a less extent in boys and men younger than 50 years of age. Infection of the urinary tract is identified by growth of significant number or a single species in the urine, in the presence of symptoms. *Escherichia coli*, followed by *Klebsiella* spp. and *Proteus* spp., cause 75-90% of all infections. Urinary culture is an accurate diagnostic method but takes several hours or days to be carried out. Metabolomics analysis aims to identify biomarkers able to speed up diagnosis. In this study, urine samples from 51 patients with previous diagnosis of *Escherichia coli*-associated UTI and from 61 healthy controls were analyzed. The <sup>1</sup>H-NMR spectra were acquired and processed. Multivariate statistical models were applied and their performances were validated using permutation test and ROC curve. An orthogonal partial least squares- discriminant analysis (OPLS-DA) shown a good separation,  $R^2Y=0.76$   $Q^2=0.45$   $p<0.001$ , between UTI samples and healthy controls. Acetate and trimethylamine were identified as discriminant metabolites. The area under the ROC curve was calculated using the predicted Y value from OPLS-DA model (0.79), using acetate (0.95) or trimethylamine (0.77), separately, or using both metabolites (0.97). The results suggest that with a metabolomics approach the urinary profile of UTIs caused by Gram- E. coli could be distinguished from that of healthy controls. Acetate and trimethylamine resulted optimal candidates as biomarkers for UTI diagnosis. The conclusions support the possibility of a fast diagnostic test for *Escherichia coli*-associated UTI by using acetate and trimethylamine concentrations.



**Poster #: 215**

**Abstract #: 2432**

**Abstract Title:** Plant-soil feedbacks introduce changes in the metabolome of common grassland species

**Authors:** Christian Ristok, Jan-Hendrik Dudenhöffer, Anne Ebeling, Nico Eisenhauer, Cameron Wagg, Nicole van Dam, Alexander Weinhold,

**Presenting Author Affiliation:** iDiv - German Centre for Integrative Biodiversity

**Abstract Submission:**

Plant-soil feedbacks (PSFs) are plant-induced changes in soil properties and organisms that feedback on the productivity and fitness of a plant. So far, the majority of studies focused on the effects of PSFs on plant biomass production. Only few studies assessed the cascading effects to higher trophic levels and illuminated the hidden mechanisms of PSF effects. It is likely that changes in the plant metabolome cause these cascading effects.

We tested this hypothesis by establishing a plant-soil feedback experiment with four perennial plant species to investigate changes in the metabolome of both above- and belowground plant organs. We grew plants in a sterile background substrate inoculated with either sterilized soil, or soil conditioned by different plant species compositions, (i) the focal plant species in monoculture, (ii) a four-plant species mixture or (iii) an eight-plant species mixture including the focal plant species. We took samples for metabolomics at the end of the flowering season and analyzed secondary metabolites by LC-qToF-MS. Data processing was performed in R statistical software using the 'xcms' and 'CAMERA' packages.

Our results indicate that species-specific aboveground and belowground metabolomes differed among the four different soil treatments. The above- and belowground metabolomes of plants grown in sterile soil overall differed from those grown in the other soil treatments. Furthermore, a variety of above- or belowground metabolites changed in concentration with increasing plant diversity of the soil's inoculum origin, while others were only found in specific soil treatments.

We conclude that soil with a history of different plant diversity levels exerts PSFs that induces changes in the plant metabolome, and that those changes in plant chemistry may be linked to important ecosystem processes, such as plant herbivory.

**Poster #: 216**

**Abstract #: 2209**

**Abstract Title:** Different metabolism of *Aspergillus oryzae* and *Bacillus amyloliquefaciens* on rice koji fermentation

**Authors:** Da Eun Lee, Sunmin Lee, Eun Seok Jang, Hye Won Shin, Dong Joo Shin, Hye Jin Kim, Byoung Seok Moon, Choong Hwan Lee,

**Presenting Author Affiliation:** Konkuk university

**Abstract Submission:**

The rice koji, used in early manufacturing process of many fermented foods, is important for product quality by producing diverse metabolites and enzymes for fermentation. Metabolite profiling of rice koji fermented with different microorganisms, *Aspergillus oryzae* (RK\_AO) and *Bacillus amyloliquefaciens* (RK\_BA), depending on fermentation times was performed by using gas chromatography time-of-flight mass spectrometry (GC-TOF-MS) and ultrahigh-performance liquid chromatography linear trap quadrupole ion trap tandem mass spectrometry (UHPLC-LTQ-IT-MS/MS) with multivariate analysis. In principal component analysis, rice koji samples was shown discriminative pattern according to inoculated microbe and fermentation time. Several enzymatic activities secreted by microorganisms including  $\alpha$ -amylase, protease, and  $\beta$ -glucosidase were investigated to reveal the different level of enzyme expression by each microbes. This approach revealed metabolisms of carbohydrate, serine-derived amino acids and fatty acids highly related with *A. oryzae* while metabolisms of aromatic and branched chain amino acids, flavonoids and phospholipids related with *B. amyloliquefaciens* due to various microbial enzyme activities on rice koji fermentation. The antioxidant activity was significantly higher RK\_BA than RK\_AO along with the total flavonoids contents including tricin, tricin glycosides, apigenin glycosides, and chrysoeriol glycosides. This study suggests that useful information to selecting inoculated microbe and optimal fermentation time in rice koji fermentation through a correlation with MS-based metabolomic approach and enzyme activity.

**Poster #: 217****Abstract #: 2320**

**Abstract Title:** Explorations of the generation of aldehydic lipid oxidation products in thermally-stressed culinary oils and fats: A <sup>1</sup>H NMR-linked PCR modelling strategy

**Authors:** Benita Percival, Sarah Moumtaz, Kerry Grootveld, Devki Parmar, Richard Odhurogu, Kavita Desai, Pim Jansson, Martin Grootveld,

**Presenting Author Affiliation:** De Montfort University

**Abstract Submission:**

**Objectives/Hypotheses:** Deleterious health effects presented by consumption of dietary lipid oxidation products (LOPs) has evoked much clinical interest. Therefore, we employed a <sup>1</sup>H NMR-linked PCR modelling strategy to explore relationships between data matrices comprising (1) aldehydic LOP concentrations generated in culinary oils/fats when thermally-stressed according to standard frying practices, and (2) the prior fatty acid contents of such frying media (FM), together with their heating times.

**Methods:** Corn, sunflower, extra virgin olive, rapeseed, linseed, canola and coconut oils, and butter and lard, were heated according to laboratory-simulated shallow-frying episodes at 180°C, and FM samples were collected at time-points of 0, 5, 10, 20, 30, 60 and 90 min. (n = 6 replicates per time-point).

Aldehydes and FM fatty acid concentrations were determined by <sup>1</sup>H NMR analysis (Bruker AV 400 MHz spectrometer). The first (dependent) PCR data matrix comprised aldehyde concentration scores vectors (PC1\* and PC2\*), whilst the second (predictor) one incorporated those from the fatty acid content/heating time variables (PC1-PC4) and their interactions.

**Results:** Trans,trans- and cis,trans-alka-2,4-dienals, 4-hydroxy-trans-2-alkenals and 4-hydroperoxy-trans-2-alkenals (phase I aldehydes predominantly arising from PUFA peroxidation) strongly/positively loaded on PC1\*, whereas n-alkanals and trans-2-alkenals (phase II aldehydes derived from both MUFAs and PUFAs) strongly/positively loaded on PC2\*. PCR analysis of these scores vectors (SVs) demonstrated that PCs 1 (positively-loaded linoleoylglycerols), 2 (positively-loaded oleoylglycerols), 3 (positively-loaded linolenoylglycerols) and 4 (exclusively positively-loaded sampling time-points) all powerfully contributed to aldehydic PC1\* SVs (p PC1) and their interactions, but not those of PC3. A Q2 value of 0.533 (R2Y = 0.810) was obtained.

**Conclusions:** NMR-linked PCR analysis is a valuable strategy for modelling the generation of aldehydic LOPs in heated FM, and also for tracking their fatty acid sources therein.

**Poster #: 218**

**Abstract #: 2253**

**Abstract Title:** Metabolomic approach for antioxidative activity of the makgeolli brewed with Korean traditional nuruk

**Authors:** HyeRyun Kim,

**Presenting Author Affiliation:** Korea Food Research Institute

**Abstract Submission:**

Makgeolli, is a Korean traditional alcoholic beverage. The taste and flavor of makgeolli are mainly determined by the metabolic products such as free sugars, amino acids, organic acids, and aromatic compounds which are produced during fermentation process of raw materials by the microorganisms present in nuruk, a Korean fermentation starter. In this study, we brewed makgeolli using 54 different nutritional ingredient-based nuruk and analyzed changes in nutritional metabolites. Though there are several studies supporting anti-cancer ( $\beta$ -sitosterol) and immune-stimulatory effect of makgeolli brewed by nuruk have been reported, the other physiological activity such as anti-oxidant effect are still unknown. Therefore, after the fermentation, antioxidative activity and changes in the metabolites were investigated by metabolomics approaches. The metabolites of makgeolli were simultaneously analyzed by liquid chromatography electrospray ionization quadrupole time-of-flight mass spectrometry (LC-ESI-Q-TOF-MS). Makgeolli metabolites profiling were affected by nutritional and microbial composition of fermentation starter. As a result, the makgeolli samples taken at different Korean traditional nuruk were clearly distinguishable in the score plot generated by combining PC1 (27.30% of the total variance) with PC2 (10.90% of the total variance). When the 10 types of makgeolli with the highest antioxidative activities were compared with the 10 types of makgeolli with the lowest, these two groups showed different metabolite profiles. The makgeolli with a high antioxidant effect showed significantly high contents of eight metabolite ingredients, including three tripeptide 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.

**Poster #: 219**

**Abstract #: 2039**

**Abstract Title:** Metabolomics and chemometric study of *Nymphaea pubescens* flower extract to identify metabolite(s) contributory to the acetylcholinesterase inhibition

**Authors:** Bratati De,

**Presenting Author Affiliation:** University of Calcutta

**Abstract Submission:**

Metabolomics and chemometric study of *Nymphaea pubescens* flower extract to identify metabolite(s) contributory to the acetylcholinesterase inhibition

Jayashree Acharya<sup>1</sup>, Mainak Dutta<sup>2</sup>, Koel Choudhury<sup>2</sup>, Bratati De<sup>1</sup>

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**ABSTRACT**

*Nymphaea pubescens* is an aquatic plant, the flowers being edible. The flowers have been reported to have many medicinal properties also. Inhibitors of acetylcholinesterase (AChE), are used presently in the treatment of Alzheimer's disease. Initial screening revealed that the flowers of *N. pubescens* inhibited AChE. This motivates us to identify the contributory metabolite(s) present in *N. pubescens* flower extracts responsible for inhibition of AChE using metabolomics and chemometric approach. The flower samples were collected from different districts of West Bengal, India in the monsoon season. Variation in their metabolite contents was analysed using Gas Chromatography - Mass Spectrometry (GC-MS) after derivatization with methoxyamine hydrochloride and N-Methyl-N-(trimethylsilyl) trifluoroacetamide. Fatty Acid Methyl Ester markers were used as internal Retention Index (RI) markers. Automated mass spectral deconvolution and identification system were used to deconvolute GC-MS and identify chromatographic peaks. The metabolite levels varied in different samples. The district wise variation in AChE inhibitory activity was also noteworthy. The metabolites as well as the activity profile were analysed by PLS-DA and OPLS-DA which differentiated between extracts with low activity (< 45% inhibition) and higher activities (> 45%). Chemometric analysis revealed gallic acid to be one of the metabolites contributing significantly towards the inhibition of AChE by the flower extracts. Of the other metabolites having correlation, shikimic acid, 4-hydroxy benzoic acid, quinic acid did not show any AChE inhibitory activity. It is suggested that they were probably involved in activity through the synergistic action. Further research is required in this regard.

**Poster #: 220**

**Abstract #: 2073**

**Abstract Title:** ASSESSMENT OF METABOLOMIC APPROACH FOR THE SCREENING OF ILLEGAL ADMINISTRATION OF CEPHALOSPORINS IN LAYING HENS

**Authors:** Murielle Gaugain,

**Presenting Author Affiliation:** Anses-Laboratoire de Fougères

**Abstract Submission:**

Monitoring of illegal veterinary treatments is for a long time based on targeted detection of administered molecules or the metabolites thereof, mainly by LC-MS/MS. The objective of this project was to look for shared biomarkers of treatments for a same class of antibiotics which could then be monitored to determine potential exposure to illegal treatment by the way of a predictive model. LC-HRMS was used for a full scan non targeted acquisition of all metabolites. Animal experimentation was performed on three groups of laying hens: control (non treated), ceftiofur and cefquinome treatment group. For each group, half of the animals were slaughtered 48h and the other half 72h after the end of the treatment. Liver, eggs and droppings were collected and rapidly stored at -80°C. A generic extraction was used in order to limit the loss of metabolites during the sample preparation process and the samples were then injected in a LTQ-Orbitrap XL system. Then, processing steps as peak-picking, grouping and alignment steps were carried out. After inter-batch normalization and removing of features having a too high analytical variability, PCA descriptive models were built before to use multivariate regression models as PLS-DA or (O)PLS-DA. The two last models allowed highlighting variables strongly involved in discrimination between treated and non-treated groups. No trend allowing differentiation between treated and non-treated animals was detected by PCA for liver or droppings. Supervised method as PLS or (OPLS)-DA enabled to show this differentiation for droppings but not for liver. For droppings, a combined analysis of univariate and multivariate results allowed choosing biomarkers in order to build and validate models including the most discriminative biomarkers. Robustness of the models will be presented via different parameters as  $R^2(Y)$ ,  $Q^2(Y)$  and number of misclassified samples.

**Poster #: 221**

**Abstract #: 2097**

**Abstract Title:** Mass spectrometry-based metabolomics to differentiate beer types

**Authors:** Shuichi Kawana, Takero Sakai, Yusuke Takemori, Daichi Yukihiro, Tsuyoshi Nakanishi,

**Presenting Author Affiliation:** Shimadzu Corporation

**Abstract Submission:**

In food science, global metabolite analysis, or 'metabolomics', is increasingly applied to a number of value-added food production areas including food-safety assessment, quality control, food authenticity, origin and processing. In this study, a mass spectrometry-based metabolomics approach was applied to differentiate and classify five types of beer (two lager beers, an ale type beer, a beer-taste alcoholic beverage and a beer-taste non-alcoholic beverage). GC-MS/MS and LC-MS/MS techniques were used to target amino acids, organic acids, sugar and nucleic acid-related compounds which are known to be the components associated with sweetness, bitterness and perceived flavor in food and beverages. Statistical analysis of the detected compounds by PCA and HCA (58 compounds detected by LC-MS/MS and 245 compounds detected by GC-MS/MS) clearly differentiated each beer type using the score plot. Beer types differed in the distribution of amino acids (proline and branched-chain amino acids), nucleosides and sugars.

By using mass spectrometry followed by principal component analysis and hierarchical cluster analysis, it was possible to successfully discriminate five beers into three major groups (beer, beer-taste alcoholic beverage and beer-taste non-alcoholic beverage).

**Poster #: 222**

**Abstract #: 2155**

**Abstract Title:** Foodomics research on the traditional Korean Nuruk and alcoholic beverages

**Authors:** Jang Eun Lee, Jae Ho Kim,

**Presenting Author Affiliation:** Korea Food Research Institute

**Abstract Submission:**

Traditional Korean Nuruk, which is a fermenter used to make alcoholic beverages out of starch, has been developed with various materials and shapes according to the geographical environments and climates of their origins. Nuruk is also known as 'kokja' in Korea, reflecting the understanding that microorganisms such as wild fungi, yeasts, and lactobacillus bacteria naturally inoculate and reproduce. Thus, it is important to identify microorganisms according to the process and quality of fermentation, and also to study the metabolites in order to understand how they affect the characteristics of the raw materials during fermentation. The objective of this study is to identify the foodomics characteristics of traditional Nuruk by recreating traditional production methods detailed in ancient Korean documents. In the present study, a total of 58 different kinds of Korean traditional Nuruk were prepared, including 46 kinds of recreated products. Foodomics research of each Nuruk as well as their alcoholic beverage products were carried out through a combination of metabolomic and metagenomic approaches. Mass and <sup>1</sup>H NMR analysis was used to identify the metabolite changes in traditional alcoholic beverages fermented with different kinds of Nuruk. In addition, microbial communities of traditional Korean alcoholic beverages were monitored during the fermentation process. This study clearly demonstrated the relationship between the microbial community and metabolites in traditional Korean alcoholic beverages fermented with Nuruk. This study also presents for the first time the Korean traditional Nuruk production methods written in ancient Korean documents, using workable production methods supported by modern technologies. Furthermore, this study analyzed the metabolomic and genomic characteristics of reproduced Nuruk, which could be utilized as a basis for further study of traditional Korean alcoholic beverages and their valuable microorganisms.



**Poster #: 223**

**Abstract #: 2325**

**Abstract Title:** Chemical Profiling of Actaea (Black Cohosh) Species Using UPLC-QToF-MS

**Authors:** Jimmy Yuk, Maged Sharaf, Giorgis Isaac, Mark Wrona, Kate Yu,

**Presenting Author Affiliation:** Waters Corporation

**Abstract Submission:**

Black cohosh (*Actaea racemosa* L.) is a native North American species, where it is widely used as an herbal supplement for the relief of menopausal symptoms. Due to the continual increases in sales and collection of *A. racemosa*, unintentional substitution or deliberate adulteration with related species were seen in the market. This has caused world-wide concerns as hepatotoxic cases are being seen. Chemical methods using analytical instrumentation such as HPTLC, HPLC with diode array, GC-MS, NMR spectroscopy, mass spectrometry have also been used to profile *Actaea* species. Many of these studies have focused on identifying key chemical components in various *Actaea* species for authentication. A newer approach has been the development of untargeted methods to compare whole chemical spectral profile using authenticated references. In the present study, a multivariate statistical method using UPLC-QToF-MS was used for the classification of different *Actaea* species and commercial black cohosh products. Principal component analysis allowed a holistic approach in showing distinct clustering between the different *Actaea* species and black cohosh commercial products. Differential analysis of results across several *Actaea* species were performed which facilitated the identification and quantitation of potential metabolite markers. Significant metabolite markers that differentiate between *Actaea* species and for black cohosh commercial products were identified. The identification of the metabolite marker was based on exact mass precursor ion, theoretical isotopic distribution, and high energy fragment ion information. Theoretical fragmentation of the candidate compounds were also performed to increase the confidence of compound identification. We identified useful metabolite marker compounds like cimifugin derivatives, triterpene glycosides and alkaloids that distinguish between the different species of *Actaea*.

**Poster #: 224**

**Abstract #: 2573**

**Abstract Title:** Systematic Integration of “Omics” Data to Improve Innovation in Beer Crafting

**Authors:** Anas Kamleh,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

Innovation is having a large impact on the beer industry, especially in microbrewery. Systems biology offers the possibility to better understand different biological interactions in yeast strains allowing the production of genetically modified strains with uniqueness in final product. We are presenting our efforts for explaining the differences between two popular beers, California Ale and Hefeweizen, using proteomic and metabolomics analyses. The fermentation process is divided into four phases known as lag, growth, fermentation and sedimentation. Ontology enrichment from the proteomic data using ProteinCenter showed, for the first time, stages in the fermentation process of both strains where cell wall synthesis is induced.

Proteomics analyses has shown cell wall synthesis induction in the different stages of fermentation. This was followed by initiation of glycolysis that leads to energy production and cell growth.

Metabolomics analyses revealed the absence of static metabolic phases. Instead, phases overlap allowing for simultaneous growth and fermentation activities. Overlapping phases are a result of the high cell heterogeneity that makes individual cells progress through fermentation at different rates. This can be observed in the time-course progression of the individual metabolites. The coefficients of variation for those metabolites increase dramatically when overlap occurs.

Metabolomics has also highlighted differences in growth rates between the two strains. This was further validated by proteomics where a cluster of ribosomal proteins involved in growth showed significant differential expressions.

California Ale has a higher attenuation compared to the Hefeweizen strain due to the different expression of alcohol dehydrogenase enzymes. We also found that at the end of the fermentation process over 300 compounds were responsible for making these beers significantly different. Overall, the combination of the two omics-based high-throughput technologies with bioinformatic tools in a systems biology framework represents a valuable approach to exploring new products in the beer industry.

**Poster #: 225**

**Abstract #: 2550**

**Abstract Title:** Classification of Beer by Mass Spectrometric Metabolite Profiling

**Authors:** Lisa M. Røst, Zdenka Bartosova, Kåre A. Kristiansen, Truls C. Rasmussen, Per Bruheim,

**Presenting Author Affiliation:** Department of Biotechnology, Norwegian University

**Abstract Submission:**

Beer brewing is a complex bioprocess. Both quality, treatment and composition of the raw ingredients water, malt, hobs, the brewing process itself, type and treatment of the yeast and the fermentation process itself, maturation and storage conditions all contribute to the development of aroma and taste of the finished product. The Beer Judge Certification Program recognizes 23 beer classes and 78 beer types, and there are strict guidelines (e.g. bitterness, color, ABV, taste and aroma profiles) for classification of beers. Thus, it is important for commercial breweries to follow the guidelines when they release new commercial products. The increasing number of craft breweries and home brewers should also pay attention to the beer type criteria, especially when participating in competitions. In this project, we have analyzed 30 commercial beers and 25 home brewed beers with various mass spectrometry methods. For the home brewed beers, we have information about yeast type, hobs and malt composition. Method selection was based on covering all of the major compound classes in the finished beer. Dynamic headspace GC-MS was used for analysis of volatile compounds (e.g. esters and alcohols), two target LC-MS/MS methods for organic acids and amino acids, an amide LC-QToF using neg ESI for sugar components, a RP LC-QToF with combi ESI/ CI ion source for analysis of bitter compounds, and a direct probe QToF method for rapid profiling of the beer. The data was processed using multivariate analysis tools to assess the variation within a beer type from commercial actors and private home brewers. The amenability of the various MS methods for classification of beers was also evaluated.

**Poster #: 226**

**Abstract #: 2363**

**Abstract Title:** Quantitative structure-retention relationships study in lipidomic analysis of grasshopper abdominal secretion (Chorthippus spp.)

**Authors:** Magdalena Buszewska-Forajta, Lukasz Kubik, Roman Kaliszan,

**Presenting Author Affiliation:** Medical University of Gdansk

**Abstract Submission:**

Classical QSRR are found as a useful tool in chromatographic experiment design based on the prediction of the analyte behaviour, which provides the best condition for appropriate separation. In this study application of QSRR in biomedical field, namely lipidomics, concerning the compounds determined in insects secretion, is proposed.

Grasshopper is known as one of the most popular insects on the Polish territory. According to ethnopharmacological observations, the mentioned above secretion, applied topically, facilitates healing of wounds and scars.

In the present work we focused on application of QSRR study in lipidomic analysis of grasshopper abdominal secretion components determined by GC/MS. Moreover the correlation between the QSRR-calculated and experimental retention indices were studied by the LASSO and LASSO/Stepwise Regression.

Grasshoppers were collected at Starogard Gdanski and Lubianka meadows. In pretreatment procedure, liquid-liquid extraction and derivatization was used. Samples were analyzed with the use of gas chromatography coupled with mass spectrometry. Obtained data were processed with the use of AMDIS software. In the fractions 38 compounds were determined for which classical 3D-QSRR approach was applied.

We obtained QSRR models basing on LASSO and LASSO/Stepwise Regression algorithms. The  $R^2$  and  $Q^2$  values for two developed LASSO model equaled 0.95 and 0.88, respectively. In case of LASSO/SR approach the  $R^2$  and  $Q^2$  equaled 0.98 and 0.67, respectively. The degrees of freedom/number of descriptors in equation was the same for both studied models, and equaled 3 (plus intercept). Moreover external validation was performed in order to verify the obtained models. In case of LASSO approach, calculated cross-validated mean squared error equaled  $1.22 \times 10^4$ , while for LASSO/Stepwise Regression it was  $1.78 \times 10^5$ . Both, LASSO and LASSO/SR, methods show similar results. However, LASSO seem to be somewhat more reliable method for creating QSRR equations.

**Acknowledgments:** This research was funded by National Science Centre 2012/07/N/NZ7/04395 grant.

**Poster #: 227**

**Abstract #: 2439**

**Abstract Title:** Integrated metabolomic, lipidomic and steroidomic profiling for revealing signatures and markers of effect induced by bisphenols exposure on human testicular function

**Authors:** Yann GUITTON, Anne-Lise ROYER, Christelle Destoits-Lethimonier, Séverine Mazaud-Guittot, Bernard Jegou, Bruno Le Bizec, Jean-Philippe Antignac,

**Presenting Author Affiliation:** LABERCA

**Abstract Submission:**

The Newplast research project (ANR-13-CESA-0012-01) focuses on substitutes and derivatives of bisphenol A (BPA) which are used in the manufacture of polycarbonate and epoxy resins, including food contact materials. Its global objective is to generate data and knowledge as regards (i) their biotransformation and biological impact on the human hepatic and reproductive functions, (ii) their modes of actions at molecular level through ligand-receptor binding / transactivation mechanisms and (iii) human external and internal exposure assessment. The effect of BPA and related substitutes (BPS and BPF) on testicular function was assessed on fetal (FEGA) and adult (TEXAS) models using an integrated metabolomic, lipidomic and steroidomic profiling approach. Three complementary workflows were then combined to generate a unique set of biological signatures as a support for markers of effect discovery. A rationalized sample preparation permitted to fractionate each characterized sample into one polar component for RPLC-ESI(+/-)-HRMS metabolomic profiling, and one apolar phase for RPLC-ESI(+/-)-HRMS lipidomic and GC-EI-MS/MS steroidomic profiling. Moreover a comprehensive characterization from both culture medium (exo-metabolome) and testicular tissue (endo-metabolome) was achieved. All together, obtained results give an extended picture of biological disruptions induced by BPA and two main substitutes (BPS and BPF) on human testicular target, including dose-responses and mixture effects. A set of annotated and particularly affected metabolites was revealed from each investigated metabolome sub-component.

**Poster #: 228**

**Abstract #: 2472**

**Abstract Title:** Application of Multi-platform Metabolomics for Characterization of the Chemical Composition of Food

**Authors:** Rosa Vázquez-Fresno, Shima Borzouie, Rupasri Mandal, David Wishart,

**Presenting Author Affiliation:** University of Alberta - The Metabolomics Innovatio

**Abstract Submission:**

More than 25,000 compounds are known as constituents of food. However, standard food composition tables only provide data of a few highly abundant chemicals macronutrients (amino acids, lipids, carbohydrates) and minerals for most fruits and vegetables. In this study, a combination of Nuclear Magnetic Resonance (NMR) spectroscopy, Liquid Chromatography- Mass spectrometry (LC-MS), Inductive Coupled Plasma (ICP)-MS, lipidomics and Gas Chromatography (GC)-MS, as well as high performance liquid chromatography (HPLC) were used to (1) assess the utility of multi-platform metabolomics for food composition analysis (2) determine the maximum degree of chemical coverage attainable by multiple quantitative metabolomic methods and (3) provide the experimental data and to make it available through a public web-accessible database ([www.foodb.ca](http://www.foodb.ca)), in the context of the Food Biomarkers Alliance (FoodBALL) project, a European Joint Programming Initiative 'A Healthy Diet for a Healthy Life'. The metabolomic-based food analysis in our multi-platform methodological study resulted in the experimental identification and quantification of more than 200 non-redundant metabolites. This includes around 40 water-soluble metabolites identified and quantified by NMR spectroscopy, 80 compounds by direct flow injection (DFI)/LC-MS/MS via Biocrates AbsoluteIDQ™ kit (comprising acylcarnitines, glycerophospholipids, sphingolipids, amino acids and biogenic amines), up to 53 trace elements by ICP-MS analysis, 48 compounds via lipidomics and GC-MS analysis (comprising glycolipids, phospholipids, cholesteryl esters, triglycerides and free fatty acids), 17 polyphenol compounds as well as 9 water- and lipid-soluble vitamins both by HPLC assays. To our knowledge, this is the largest number of food compounds identified and quantified in a single study using metabolomics-based food analysis. Moreover, all the analytical methods used in this study were demonstrated to be complementary, suggesting that a multi-platform metabolomics analytical procedure should be routinely used for the characterization of the chemical composition of foods in order to achieve vast coverage of the metabolome in food analysis research.

**Poster #: 229**

**Abstract #: 2369**

**Abstract Title:** Metabolomics reveals perturbations in the metabolome of endometriosis women

**Authors:** Koel Chaudhury, Mainak Dutta, Mamata Joshi, Sudha Srivastava, Swagata Dasgupta, Baidyanath Chakravarty,

**Presenting Author Affiliation:** School of Medical Science and Technology, Indian I

**Abstract Submission:**

Endometriosis is a common benign gynecological disease, characterized by growth and proliferation of endometrial glands and stroma outside the uterus. With studies showing metabolic changes in various biofluids of endometriosis women, we have set upon to investigate whether endometrial tissue show differences in their metabolic profiles. Women confirmed with endometriosis (n=126) were classified into various stages (Stage I= 20 Stage II= 16 Stage III= 45 and Stage IV= 45). Fifty seven patients undergoing interval tubectomy were included as controls. <sup>1</sup>H NMR analysis was performed on eutopic endometrial tissue of women with endometriosis and controls. Analysis was performed on spectral data and on relative concentrations of metabolites obtained from spectra using multivariate and univariate data analysis. Analysis shows that various energy, ketogenic and glucogenic metabolites have significant altered concentrations in various stages of endometriosis. This study also attempts to validate our earlier findings of serum metabolite markers in a new cohort of patients. In addition, the validated serum markers are correlated with the corresponding tissue metabolite profile of women with minimal/mild stage of the disease to ascertain their role for early diagnosis of endometriosis. While alanine (p<0.0001), lysine (p=0.0017), phenylalanine (p=0.005) and leucine (p=0.01) showed negative correlation, proline (p=0.001) showed positive correlation between tissue and serum levels of early endometriosis. The differentially expressed metabolites found in this study provide an important first step towards developing effective bioassays for diagnosing endometriosis. The scientific findings are expected to assist clinicians in better management of women with the disease.

**Poster #: 230**

**Abstract #: 2380**

**Abstract Title:** Systems biology of circadian clock signaling in *Neurospora crassa*

**Authors:** Michael Judge, Ricardo Borges, James Griffith, Jonathan Arnold, Art Edison,

**Presenting Author Affiliation:** University of Georgia

**Abstract Submission:**

Multiple lines of evidence point to an intercellular signaling mechanism for the synchronization of circadian clocks in eukaryotic microbes at the single cell level. For instance, computational models of a genome-wide circadian clock gene network built using transcriptomic data from our group have predicted an important role for an entrainment-independent synchronization mechanism in *Neurospora crassa*, and groups of single cells in liquid media have clocks more synchronized than those cells that are isolated. We are generating direct evidence for synchronization of both millions of cells and at the single-cell level using fluorescence markers in a microfluidics device. We are also isolating the signaling molecule(s) and identifying the major genes in the underlying network responsible for these signaling interactions. These goals are being accomplished using genetic screens on knockout libraries and time series NMR-MS exometabolome experiments on wild-type and synchronization-deficient/-enhanced *N. crassa* mutants from the screen. A mutant with increased single-cell synchronization has already been identified several exometabolite circadian features have been measured using NMR and MS, and a high-throughput assay has been developed for genetic screening and bioactivity-guided signal isolation. Lists of synchronization-related genes and metabolites will be obtained, and molecules in the pathways in the intersection between them will be used in high-resolution NMR-MS studies to identify the signal. This work offers fundamental insights about inputs and outputs of a pervasive systems behavior on multiple biological levels in a powerful model organism.



**Poster #: 231**

**Abstract #: 2017**

**Abstract Title:** Skin and plasma lipid profiling for the evaluation of the nutricosmetical effect of borage oil diet in healthy human subjects

**Authors:** Kwang-Hyeon Liu, Jong-Cheol Shon, Yunhi Cho, Choong Hwan Lee,

**Presenting Author Affiliation:**

**Abstract Submission:**

Borage oil (BO) having the rich  $\gamma$ -linolenic acid [GLA 18:3(n-6)] is known to be efficacious in treating skin disorders. To examine the effects of borage oil on the skin and plasma lipid profiles, borage oil was administered on human healthy subjects for 10 weeks. The placebo group (CON) received soybean oil (SO). Skin and serum lipid metabolites were analyzed using direct infusion nanoelectrospray-ion trap mass spectrometry and LC-MS/MS. BO and CON administered groups were clearly discriminated from each other on partial least squares-discriminant analysis (PLS-DA) score plot through plasma lipid profiling, and major metabolites contributing to the discrimination were assigned as triglycerides (TGs), phosphatidylcholines (PCs), and phosphatidylethanolamines (PEs). TG levels were decreased in BO group, whereas PC and PE levels were increased. However, ceramide profiles in stratum corneum of BO ingested subjects has not significantly changed compared to the CON group. These results indicate that borage oil has a significant effect on the plasma lipid profiles. This study was supported by a grant of the Korean Health Technology R&D Project, Ministry of Health & Welfare, Republic of Korea (HN13C0076).

**Poster #: 232**

**Abstract #: 2098**

**Abstract Title:** What Are We Eating? Differential Metabolomic Profiles Reveal an Insight into our Dietary Habits

**Authors:** quentin enjalbert, Paul Clemens, Baljit Ubhi,

**Presenting Author Affiliation:** Sciex

**Abstract Submission:**

Markers of the potatoes from the French fries in the American food plate as well as markers of green tea from the Californian food plate and soy-bean constituents from the Davis plate were amongst the largest signals discovered from the reverse phase Metabolomics experiments. Most of the other signatures found were from the many ingredients which made up the food items on each plate. These also included many fruit, vegetable and fish metabolites as well as spices, herbs and flavorings. Interestingly we found a red cabbage metabolite in the negative data from the reverse phase analysis which was blinded to us in the original study. Many pesticides in the reverse phase data were also found. From the HILIC data the largest differentiator was caffeine – on evaluation of the data we found more caffeine in green tea (from the Californian food plate) than in a can of soda (from the American food plate). From the lipid analysis many lipid molecular species common to fish, potato and eggs were found. From the Lipidizer Platform results we could quantitate not just total lipids at the class level, but also at the molecular species level as well as evaluating fatty acid composition. This gives us insight into how fatty acid metabolism is changing and which lipids are coming from the diet and which are being created naturally by the body and the impact of each. These studies clearly highlight that what we eat directly impacts our metabolism and that we can clearly differentiate healthier diets from more “fatty” diets.

**Poster #: 233**

**Abstract #: 2196**

**Abstract Title:** MS-based metabolomic evaluation of traditional *Rubus coreanus* vinegar and its antiosteoporotic prophylactics in ovariectomized rat models

**Authors:** Mee Youn Lee, Yu Kyung Jang, Digar Singh, Hyang Yeon Kim, Soo Hwan Yeo, Seong Yeol Baek, Yoo Kyoung Park, Choong Hwan Lee,

**Presenting Author Affiliation:** Konkuk University

**Abstract Submission:**

Vinegar is a sour traditional fermented condiment with various health benefits including immunomodulatory, anti-obesity effects. In this study, we assessed the comprehensive metabolite profiles coupled with antioxidant activity evaluation from the 2-traditional: *Rubus coreanus* (RC) and *Rhus verniciflua* (RV), and 7-commercial vinegar types. Non-targeted and targeted metabolite profile of vinegars were performed by gas chromatography-time of flight mass spectrometry (GC-TOF-MS) and ultra-performance liquid chromatography quadruple-time-of-flight mass spectrometry (UPLC-Q-TOF-MS) with multivariate statistical analyses. The proportionally higher concentrations of cyanidin-3-xylosyl rutinoside, cyanidin-3-rutinoside, and quercetin in RC vinegar rendered it superior antioxidant activity. Further, the metabolomic vista of RC vinegar consumption from 5-ovariectomized rat groups: sham, ovariectomized (OVX) rats without treatment, low-dose RC vinegar (LRV)-treated OVX rats, high-dose RC vinegar (HRV)-treated OVX rats and alendronate (ALN)-treated OVX rats, were investigated. The health efficacy for RC vinegar was gauged using physical, biochemical, and histological parameters with their positive correlations among the LRV and HRV groups, indicating the estrogen regulation. The plasmatic titers of anti-osteoporosis biomarkers including butyric acid, phenylalanine, glucose, tryptophan and some lysophosphatidylcholines were detected comparatively higher among LRV and HRV rat groups which further envisages the potential bone health prophylactic for RC vinegar. The study emphasizes the health benefits of traditional dietary RC vinegar as a part and parcel in dietary cuisines.

**Poster #: 234**

**Abstract #: 2243**

**Abstract Title:** Metabolomics Approach for the Authentication of Various Botanicals and Dietary Supplements Using UPLC/QToF-MS

**Authors:** Giorgis Isaac, Bharathi Avula, Yanhong Wang, Jimmy Yuk, Mark Wrona, Kate Yu, Ikhlas Khan,

**Presenting Author Affiliation:** Waters Corporation

**Abstract Submission:**

Metabolomics can be used to provide an unbiased, comprehensive qualitative and quantitative overview of the metabolites present in botanicals and dietary supplements. Compared to conventional analyses which are focused on a limited set of compounds, metabolomics approaches, together with novel data processing tools, enable a more holistic comparison of samples. The metabolite profiles of botanicals (such as Hoodia, Terminalia and chamomile) and their commercial products were investigated to determine the clustering pattern and identification of different metabolites. The botanicals and corresponding commercial products were randomized and injected three times with a set of QC pooled sample runs in both positive and negative ion mode. Details of data file format and a list of expected adducts are entered to facilitate the handling of data import followed by automatic retention time alignment. Metabolomics experiments involve large amount of sample runs that may result in a shift in retention time. The LC/MS data was first aligned to correct any retention time drift between analytical runs. After retention time alignment, automatic peak detection, normalization, deconvolution, compound quantitation, identification and statistical analysis was performed. Principal component analysis (PCA) was performed and different groups were separated on the basis of the PCA analysis, reflecting the botanical species and corresponding commercial products. Differential analysis of results across several botanical species can quickly be performed. Significantly changing metabolite markers that differentiate between various botanical species and commercial products were identified that can be used as a target markers for botanical authentication. The identification of the marker metabolites was based on exact mass precursor ion, theoretical isotopic distribution, retention time and high energy fragment ion information. To improve the confidence in the compound identification, theoretical fragmentation of a candidate compounds was performed and then matched to the resulting 'in silico' fragmentation against the measured fragments for a compound.

**Poster #: 235**

**Abstract #: 2354**

**Abstract Title:** The ETD-Like Fragmentation for Small Molecules

**Authors:** Tim Stratton, Romain Huguet, Mark Berhow, Chad Weisbrod,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

Flavonoids are a class of plant and fungus secondary metabolites (three-ring structure C6-C3-C6 with various substitutions) involved in many functions such as pigmentation, UV filtration, symbiotic nitrogen fixation, cell cycle inhibitors and defense mechanisms. These polyphenolic antioxidants have been incorporated for thousands of years in Eastern medicine but have yet to be utilized in Western therapeutics despite their phenomenal record in providing health benefits. For example, regular consumption of flavonoids may reduce the risk of death from coronary heart disease for elderly people. An increasing number of studies today are investigating their biological properties, many with a focus on the connection between their antioxidant activity and their chemical structures.

In this study, we performed direct infusion experiments of various flavones and conjugated flavonoids with a modified Orbitrap Fusion Lumos Tribrid™ mass spectrometer. We performed CID, HCD and UVPD fragmentation experiments for structural investigation.

The data acquired from the different fragmentation techniques were used to reveal the molecular structures of each flavonoid using mzCloud™ and Mass Frontier™. The preliminary tests performed with the flavone Robinin showed that UVPD experiments provided much more informative MS/MS spectral information than the other dissociation techniques. While there were many fragment ions in common between techniques, UVPD also provided unique fragmentation channels in both low and high mass range: 121.0283, 149.0231 and 763.204. These preliminary results indicate the potential for UVPD as a tool in the structural elucidation of conjugated flavonoid structures especially as CID and HCD fragmentation techniques are severely limited for flavonoid glycoside characterization. The diversity of chemical space in conjugated flavanoids is extremely large due to combinations of conjugate sugars on multiple flavonoid cores and different linking positions. UVPD definitely offers the ability to gain unique fragmentation information which could lead to the position of conjugation that could be the key for identification.

**Poster #: 236**

**Abstract #: 2610**

**Abstract Title:** The Lipidome in Weight Loss

**Authors:** Rachel Spicer, Larissa Richardson, Jules Griffin, Reza Salek, Christoph Steinbeck,

**Presenting Author Affiliation:** EMBL-EBI

**Abstract Submission:**

In the UK 64% of adults are now either overweight or obese. Referral to commercial programs for weight management has been found to be an effective treatment in previous trials,. During the weight loss referrals for adults in primary care (WRAP), 1200 overweight and obese participants, from 3 centres across the UK, were randomly assigned to three treatment groups: a standardised brief intervention (BI) or a referral to a commercial programme for 12 (CP12) or 52 weeks (CP52). Participants allocated to the commercial treatment groups received vouchers to attend Weight Watchers sessions for either 12 or 52 weeks those in the BI group were provided with a booklet on self help weight loss strategies. Fasting blood samples were then taken from participants at 0 and 12 months and blood plasma was extracted. Lipids were recovered from these samples using methyl-tert-butyl (MTBE) extraction. Following extraction, samples were then analysed using direct infusion mass spectrometry (DI-MS). This study aims to identify changes in individuals' lipidome profile composition between the inception of the study and a 12 month follow up appointment, as well as differences between the intervention groups.

**Poster #: 237**

**Abstract #: 2471**

**Abstract Title:** Using metabolomics approaches to explore the potential disease-causing mechanism of cooking oils: a short-term human feeding study

**Authors:** Po-Sheng Wang, Wen-Harn Pan,

**Presenting Author Affiliation:** National Taiwan University

**Abstract Submission:**

Fats and oils play important roles in maintaining human nutrition and health through providing energy, essential fatty acids, and acting as modulators of many biological processes (signal transduction, immunity and inflammation). Due to differences in the fatty acid composition and content of antioxidants of individual cooking oils, the biological effect may vary oil by oil. Therefore, the purpose of this study is to compare the human response to several popular cooking oils which has different fatty acid composition using untargeted metabolomics. The study is a human feeding study which provide milkshakes with six kinds of testing oils (control without oils, soybean oil, olive oil, palm oil, camellia oil and tallow) to 15 healthy man. Fasting and post-prandial serum (2 and 4 hour) samples have been collected to measure the change in metabolomics profile.

The study results showed that the level of metabolites related with lipid digestion and lipid metabolism are significantly increased after cooking oil consumption. In addition, the intensity of metabolites related with neurotransmitter, purine and pyrimidine metabolism, anti-oxidative and amino acid metabolism was higher in the oil-intake group than control group, there were also difference between various kind of cooking oil group. The subjects intake different cooking oil had a different physiological metabolites profiling and therefore affected human health.

**Poster #: 238**

**Abstract #: 2008**

**Abstract Title:** Nutritional metabolomics: A serum based metabolomics strategy for determining dietary flavanoid intake in human adults

**Authors:** Lee Gethings, David Charles, Peter Burney, Vanessa Garcia-Larsen,

**Presenting Author Affiliation:**

**Abstract Submission:**

Flavonoids, abundant in fruits and vegetables, a family of compound with a benzo- $\gamma$ -prone core, have been shown to exert several functions (including antioxidant and anti-inflammatory) which might protect against allergy, and inflammation and oxidative stress in the airways. Here, we report a detailed metabolomic study in serum from a sample of adults from the UK taking part in the Global Asthma and Allergy Network of Excellence (GA2LEN) Follow-up survey. Serum samples were treated with methanol and centrifuged for 15 minutes. The remaining sample was aliquoted from the precipitated protein pellet and dried overnight using a vacuum evaporator. Samples were reconstituted using water with 0.1% formic acid prior to LC-DIA-MS analysis. Data was processed and searched against the Human Metabolite Database (HMDB) and ChemSpider using Progenesis Q1. In total, 46,000 features were peak picked and normalised. Unsupervised multivariate statistical analysis of the resulting data showed clear separation between the two dose groups. Supervised OPLS discriminate analysis was used to filter for features of significant correlation and covariance prior to database searching. A total of 1,200 features were isolated as being of high covariance and correlation of which 304 features had identifications appended. Identifications matching criteria as follows, mass accuracy 2 were considered for further interrogation. Normalized label-free quantitation results highlighted differential expression of specific compound classes including flavan-3-ols, anthocyanins, flavones and proanthocyanidins. Comparison of the LC-MS data with previous epidemiologic studies and the FFQ showed an association for various flavanoid metabolites identified by LC-MS. In particular, good correlation for several flavanoid subclasses examined included salviaflaside methyl ester, 1,2,8-trihydroxy-3-methylanthraquinone and rubraflavone A which indicated Spearman's rank correlation coefficients  $>0.5$ .



**Poster #: 239**

**Abstract #: 2037**

**Abstract Title:** Lipidomics Analysis Reveals the Pharmacological Effects of Platycodon grandiflorum extracts in High-Fat Diet Induced Obese Mice

**Authors:** Kab Tae Park,

**Presenting Author Affiliation:** College of Pharmacy and Research Institute of Phar

**Abstract Submission:**

The roots of *Platycodon grandiflorum* (PG) have a variety of pharmacological properties, including anti-obesity properties. The aim of this study was to investigate the clinical changes induced by PG extracts consumption in a high-fat diet (HFD) mice model. To investigate the lipid metabolites that affected the change of the clinical factors, the serum and liver of mice fed a normal control diet (NCD), HFD, HFD plus PG 1% diet (HPG1), and HFD plus PG 5% diet (HPG5) were analyzed using direct infusion nanoelectrospray-ion trap mass spectrometry combined with multivariate analysis. The NCD, HFD, HPG1, and HPG5 groups were generally discriminated upon principal component analysis (PCA) and partial least-squares discriminant analysis (PLS-DA) score plot, and the major metabolites contributing to such discrimination were triglycerides (TGs), cholesteryl esters (CEs), phosphatidylethanolamines (PEs), phosphatidylcholines (PCs), and lysophosphatidylcholines (LPCs). We also found that PC and TG levels differed substantially according to the length of the acyl chain and number of total double bond, respectively. When the PG 5% extracts were fed to obese mice, the levels of metabolites including some of the phospholipids (LPCs, PCs, and PEs) became similar to those of mice fed a normal control diet. Such metabolic markers can be used to better understand obesity and related diseases induced by a hyperlipidic diet. In this study, PG extracts might potentially inhibit HFD-induced obesity and changes in the levels of such lipid metabolites can be employed to assess the risk of obesity

**Poster #: 240**

**Abstract #: 2063**

**Abstract Title:** Metabolic profiles from two different breakfast meals characterized by <sup>1</sup>H NMR-based metabolomics

**Authors:** Millie Rådjursöga, Göran Karlsson, Helen Lindqvist, Anders Pedersen, Cecilia Persson, Rui Pinto, Lars Ellegård, Anna Winkvist,

**Presenting Author Affiliation:** Department of Internal Medicine and Clinical Nutri

**Abstract Submission:**

It is challenging to measure dietary exposure with techniques that are both accurate and applicable to free-living individuals. We performed a cross-over intervention, with 24 healthy individuals, to capture the acute metabolic response of a cereal breakfast (CB) and an egg and ham breakfast (EHB). Fasting and postprandial urine samples were analyzed using <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy and multivariate data analysis. Metabolic profiles were distinguished in relation to ingestion of either CB or EHB. Phosphocreatine/creatine and citrate were identified at higher concentrations after consumption of EHB. Beverage consumption, i.e., tea or coffee, made a substantial part of the model. 2-furoylglycine and 5-hydroxymethyl-2-furoic acid - a potential biomarker for coffee consumption, were identified at higher concentrations in coffee drinkers. Thus <sup>1</sup>H NMR urine metabolomics is applicable in the characterization of acute metabolic fingerprints from meal consumption and in the identification of metabolites that may serve as potential biomarkers.

**Poster #: 241**

**Abstract #: 2266**

**Abstract Title:** Comparison of statistical methods to analyze longitudinal metabolomics data

**Authors:** Marie Tremblay-Franco, Cécile CANLET, Aurélien AMIEL, Isabelle SAVARY-AUZÉLOUX, Didier REMOND, Sergio POLAKOF,

**Presenting Author Affiliation:** INRA ToxAlim

**Abstract Submission:**

Dynamic metabolomic analysis gives new insights into metabolic modulation (or dysfunction) after diet challenges. However experimental designs involving the study of dynamic changes raise new methodological challenges in the field of data analysis. This study aims at comparing the Anova-Simultaneous Component Analysis and the multilevel approach to assess post-prandial modulation of plasma metabolism in minipigs.

Multicatheterized minipigs were submitted to a high fat diet during two months. Plasma samples from major hepatic vessels (artery, portal vein and hepatic vein) were collected before the high fat diet (J0), and after 7 (J7) and 60 (J60) days of feeding. For each date, samples were collected at 0, 1, 3, 5.5 and 8.5 hours after meal intake. Plasma samples were analyzed by <sup>1</sup>H NMR spectroscopy. NMR spectra were phased and baseline corrected, and data were reduced to integrate 0.01 ppm wide regions within the 10.0-0.5 ppm region.

Anova-Simultaneous Component Analysis (Jansen et al. 2005) combines ANOVA and SCA: data are first separated into blocks corresponding to the different sources of variation (experimental design factors). Then PCA is independently applied on each block, and permutations test is used to evaluate the significance of model parameters. Multilevel approach (Liquet et al. 2012) splits variation in two parts: within and between subject variation. Then multivariate method such as sparse Partial Least Squares regression (Lê Cao et al. 2009) can be used to analyze the within variation.

A-SCA and Multilevel methods were applied to data collected at J60. Both methods enabled to discriminate data collected before and after meal intake. On the other hand, samples from artery (liver input) and samples from the hepatic vein (liver output) were separated.

In this experimental design, A-SCA and Multilevel sPLS-DA lead to the same results.

**Poster #: 242**

**Abstract #: 2334**

**Abstract Title:** Strawberry consumption alters the urinary metabolome of smokers and nonsmokers

**Authors:** Matthew Teegarden, Morgan Cichon, Jessica Cooperstone, Jennifer Ahn-Jarvis, Christopher Weghorst, Yael Vodovotz, Steven Schwartz,

**Presenting Author Affiliation:** The Ohio State University

**Abstract Submission:**

The U.S. Surgeon General recognizes causal relationships between cigarette smoking and a host of chronic diseases, including oral-pharyngeal cancer and cardiovascular disease. In total, smoking is associated with almost 500,000 deaths and billions of dollars in healthcare costs per year. Promising results from clinical research support a potential role for berries and their associated phytochemicals in the prevention of smoking related diseases. Strawberries are a relatively accessible source of berry phytochemicals but little is known how smoking may affect their absorption and metabolism. Our group recently conducted a randomized, cross-over, placebo-controlled clinical study, in which smokers and non-smokers consumed a novel confection containing lyophilized strawberry powder (LSP, 24 g/day) and placebo confections, each for 7 days. A difference in 24 hr urolithin A excretion between smokers and non-smokers trended towards significance ( $p=0.078$ ), suggesting that smoking may affect berry phytochemical metabolism. The objective of this work was to further investigate potential differences in the urinary metabolomes of smokers and non-smokers following a strawberry intervention using an untargeted approach. Urine samples from 9 smokers and 10 non-smokers following the placebo and strawberry interventions were volumetrically normalized according to osmolality and profiled using UHPLC-QTOF-MS. Data were analyzed using both univariate and multivariate techniques. Over 70 features were found to be significant between the treatments ( $P<0.05$ ). To capitalize on the crossover design of the clinical trial, a multilevel approach was used for the multivariate data analysis. Using principal component analysis, the majority of the variation between subjects was found to be explained by smoking status. When exploring variation associated with the treatment conditions, subjects were clearly differentiated based on treatment with possible evidence for differences between smokers and non-smokers. These results will inform future work on the prevention of smoking-related diseases with dietary interventions, such as berries.

**Poster #: 243**

**Abstract #: 2408**

**Abstract Title:** Plasma biomarkers in Parkinson's disease

**Authors:** Olimpio Montero,

**Presenting Author Affiliation:** Spanish Council for Scientific Research (CSIC)

**Abstract Submission:**

Plasma biomarkers in Parkinson's disease

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**ABSTRACT**

Parkinson's disease (PD) is the second important neurodegenerative disease worldwide. Accurate methods for early diagnoses with minimal invasion are highly demanded. In this study, plasma samples from i) idiopathic Parkinson's disease patients diagnosed de novo with no dopaminergic treatment (PDOFF), ii) idiopathic Parkinson's disease patients diagnosed and with dopaminergic treatment (PDON), and iii) healthy controls (C), were analyzed by ultrahigh performance liquid chromatography coupled to mass spectrometry (UPLC-ESI-QToF-MS) with the aim to find potential biomarkers for early and differential diagnoses of Parkinson's disease. Levels of  $\alpha$ -Synuclein in the plasma samples were also measured using specific antibodies by ELISA. Data acquired by UPLC-MS were submitted to untargeted analysis by partial least-square discriminant analysis (PLS-DA) using MarkerLynx<sup>®</sup> and Extended Statistics (XS)<sup>®</sup> software. No clear groups could be distinguished in the Score-Plots when all the samples were compared, but PDOFF and PDON samples could be grouped separately from control samples (C) after paired comparison. C20:5-lysophosphatidylcholine (LPC(20:5)) was decreased in PDOFF group as compared to controls, which could suggest reduced activity of the phospholipase iPLA2<sup>?</sup> conversely, propionyl-carnitine and C18:2-carnitine were augmented in PDOFF group as compared to controls but not in PDON group, this fact suggesting an alteration in  $\beta$ -oxidation metabolism in sick subjects that is at least partially corrected in treated subjects. As well, phenylpyruvic acid and phenylacetylglutamine were increased in PDOFF group but not in PDON group as compared to controls these metabolites are directly associated to phenylalanine and DOPA metabolisms.

**Poster #: 244**

**Abstract #: 2419**

**Abstract Title:** Meat, the metabolites: an integrated metabolomics and lipidomics approach for the detection of meat adulteration

**Authors:** Drupad Trivedi, Katherine Hollywood, Nicholas Rattray, Holli Ward, Dakshat Trivedi, Joseph Greenwood, David Ellis, Roy Goodacre,

**Presenting Author Affiliation:** University of Manchester

**Abstract Submission:**

Adulteration of high quality food products with sub-standard and cheaper grades is a world-wide problem taxing the global economy. Currently, many traditional tests suffer from poor specificity, highly complex outputs and a lack of high-throughput processing. Metabolomics has been successfully used as an accurate discriminatory technique in a number of applications including microbiology, cancer research and environmental studies and certain types of food fraud. In this study, we have developed metabolomics as a technique to assess the adulteration of meat as an improvement on current methods. Different grades of beef mince and pork mince, purchased from a national retail outlet were combined in a number of percentage ratios and analysed using GC-MS and UHPLC-MS. These techniques were chosen because GC-MS enables investigations of metabolites involved in primary metabolism whilst UHPLC-MS using reversed phase chromatography provides information on lipophilic species. With the application of chemometrics and statistical analyses, a panel of differential metabolites were found for identification of each of the two meat types. Additionally, correlation was observed between metabolite content and percentage of fat declared on meat products' labelling.

**Poster #: 245**

**Abstract #: 2221**

**Abstract Title:** Precise Molecular-Feature Analysis (PMA) of UHPLC/Q-TOF MS for Metabolite Profiling in Synthetic Biology

**Authors:** Ying Wang,

**Presenting Author Affiliation:** Novartis Institutes for Biomedical Research

**Abstract Submission:**

The secondary metabolites of microorganisms are biosynthesized in genetically encoded pathways, which are clustered with regulatory and resistance genes in microbe genomes. Under laboratory conditions, most of gene clusters are usually not expressed. Therefore, the “silent” pathways represent a valuable and unexploited reservoir of new metabolites for drug discovery. We use three strategies to activate these pathways, including extended cultivation, native host engineering and heterologous host expression. To meet the challenges of metabolite profiling in synthetic biology, we have developed Precise Molecular-feature Analysis (PMA) of UHPLC/Q-TOF MS. High resolution MS, isotope pattern, ion charges, molecular-feature algorithm and retention time are used together in PMA to get cleaned mass spectra for precise identification and comparative quantification. Process automation with large UHPLC/Q-TOF MS data sets is realized by scripting with VBS. In non-targeted analysis of engineered native hosts in various media, PMA-MS libraries are first established with all metabolites of wild-type samples. In the comparative metabolite profiling, new compounds biosynthesized by activated unknown gene clusters in engineered native hosts can be precisely identified in comparison with PMA-MS libraries in a fully automatic way. For the known biosynthetic pathways in heterologous host expression, PMA-MS libraries with targeted metabolites are simulated based on adduct type, ion charge state, high resolution MS, isotope pattern and peak width (FWHM). The metabolites from heterologous expression samples can be quickly found by direct comparison with simulated PMA-MS libraries in targeted analysis. In the PMA process, molecular-feature algorithm is used as the second dimension in separation, in addition to UHPLC. This two dimension strategy has significantly increased the separation performance and decreased the background noise influence. PMA-guided quantification and preparative separation has also been implemented for reproducibility monitoring, as well as for getting targeted metabolites in the natural product drug discovery driven by synthetic biology.

**Poster #: 246**

**Abstract #: 2435**

**Abstract Title:** Investigation of metal-catalyzed regioselective H/D exchange as an efficient and flexible tool to produce stable isotope labelled compounds for quantitative metabolomics

**Authors:** Annelaure Damont, Sophie Feuillastre, Grégory Pieters, Christophe Junot, François Fenaille,

**Presenting Author Affiliation:** CEA Saclay

**Abstract Submission:**

In the recent years, the extensive use of liquid chromatography coupled with mass spectrometry detection (LC/MS) for metabolomics applications has demonstrated the power of this bioanalytical tool in the analyses of complex mixtures originating from human or animal bio-fluids, plant extracts or even environmental samples.

Quantitative LC/MS analyses of relevant metabolites in complex matrices are crucial in metabolomics to better understand biological processes. In targeted metabolomics, stable isotopically labelled internal standards are considered essential for the determination of absolute metabolites amounts. However, the commercial availability of such stable isotope labelled standards is limited and their costs are often prohibitive.

Thus, there is an urgent need for the development of synthetic methods allowing rapid, efficient and reliable preparation of stable isotope labelled standards dedicated to large-scale metabolomics. To avoid the laborious approach of total re-synthesis of labelled compounds, we focused on direct labelling strategies and more especially on H/D exchange processes. These processes involve metal-catalysed reactions. Ruthenium-catalysed reactions are particularly appealing since they are well-suited for the alpha-deuteration of amines and alcohols, and that a large number of metabolites feature such functional groups in their structure.

The objective of this study was to achieve selective H/D exchange and high deuterium incorporation on a number of model metabolites. The labelling of amino acids mixtures was first investigated and showed very high selectivity for H/D exchange at carbon atoms bearing an amine or alcohol function (> 95 %), with an average 85-90 % deuterium incorporation efficiency. The resulting deuterated compounds were used as internal standards for the quantitative monitoring of amino acids in biological samples by LC/MS. The extension of such a process to the labelling of a broad set of metabolites directly from complex biological matrices will also be discussed.



**Poster #: 247**

**Abstract #: 2470**

**Abstract Title:** Obtaining component NMR spectra through calculation – Isolation not required

**Authors:** Jan Schripsema, Sonia Maria da Silva, Jaine Luiz, Anelique Almeida, Denise Dagnino,

**Presenting Author Affiliation:** Universidade Estadual do Norte Fluminense

**Abstract Submission:**

Typically, in metabolomics series of data are obtained from numerous samples, which contain similar components. In this paper we show that in the analysis of the data cross correlations can be made and it is possible to extract individual component spectra from the crude spectra through calculation.

The composition of essential oils is traditionally determined by GC-MS, but to a limited extent the fingerprinting by <sup>13</sup>C NMR has been reported. Here, we report the analysis of the essential oils from basil (*Ocimum basilicum* L.) and vetiver grass (*Cymbopogon zizanioides* L.). For basil a number of chemotypes has been reported, containing different proportions of monoterpenes, sesquiterpenes and phenylpropanoids. Vetiver grass produces a highly valued oil which is reported to be present in about 90% of all western perfumes. Its composition is extremely complex containing hundreds of components, mainly sesquiterpenes.

From each of the essential oil samples the following data were obtained: <sup>1</sup>H and <sup>13</sup>C NMR spectra and GC-MS analysis data.

The major components of the basil samples were found to be linalool, eugenol, camphor, eucalyptol, germacrene D and fenchone. In the vetiver samples khusimol was generally seen as major component, but at a relative low percentage of up to about 10%. Several commercial vetiver samples were found to be adulterated. By calculations on the datasets, pure NMR spectra of individual component were obtained directly from the crude mixture spectra.

It was possible to obtain the calculated pure spectra from compounds present at percentages down to 1-2 percents of the total sample. We expect that with further development of software the crude NMR spectra can be decomposed in individual compound spectra, without the need of external databases.

**Poster #: 248**

**Abstract #: 2493**

**Abstract Title:** FlavonoidSearch for high-throughput detection of flavonoids

**Authors:** Nayumi Akimoto, Kunihiro Suda, Chiaki Ikeda, Daisuke Nakajima, Hideyuki Suzuki, Daisuke Shibata, Nozomu Sakurai,

**Presenting Author Affiliation:** Kazusa DNA Research Institute

**Abstract Submission:**

A highly accurate and highly specific discrimination system of flavonoids was developed for liquid chromatography-mass spectrometry (MS) based metabolomics. Flavonoids are compounds derived from secondary metabolisms in plants, and more than 7000 structures have been reported so far including those of modified by fermentation and in animal bodies. Many of them show bioactivities such as anti-oxidative, anti-cancer, estrogen-like, and signal transduction activities, and their effects on human health are extensively studied in recent years. However, only a limited number of flavonoids can be identified by MS-based metabolomics. Major reasons of this are limited availability of standard compounds and hence insufficiency of MS/MS fragmentation data as references. By constructing a database of virtual MS/MS fragments that are expected from known flavonoid structures, we developed a flavonoid prediction system named "FlavonoidSearch." Fragmentations of flavonoids largely affected by substituents attached to the C6-C3-C6 backbone structure. Therefore, more than 3600 unique structures that were expected to cleave with the backbone were manually examined to create probable MS/MS fragments using expertise of a researcher who was versed in MS/MS fragmentation of flavonoids found in a vast amount of literature. We also used some heuristics of fragmentation which we found in measurements of standard compounds. A Java tool to query the fragment data to the database was also developed. The tool showed the highest accuracy of flavonoid identification compared to the other existing tools. The hit score of the tool showed a good index to discriminate flavonoids from other compound classes in a high specificity ( $> 0.9$ ). We found many candidates of novel flavonoids in a daily consumed dietary plant, parsley, using a list of substituents we prepared during the construction of the virtual MS/MS database. We believe that FlavonoidSearch should help to depict the details of the flavonoid world and further use of their functions.

**Poster #: 249**

**Abstract #: 2061**

**Abstract Title:** Real-time monitoring of p53's effects on pyruvate metabolism in live mitochondria using in-organelle NMR metabolomics

**Authors:** WENJUN XU,

**Presenting Author Affiliation:** Seoul National University

**Abstract Submission:**

Recent studies point out the link between altered mitochondrial metabolism and cancers, but metabolic monitoring of live mitochondria has been hampered by the lack of a proper method. p53, a well-established tumor suppressor, is also increasingly recognized as a metabolic modulator. We propose in-organelle NMR metabolomics, through which we profiled p53's metabolic effects on live mitochondria in real-time. The results suggested previously unknown roles of p53 in maintaining mitochondrial pyruvate consumption and lactate production. We also show that lactate synthesized in mitochondria is exported to cytosol. Subsequent experiments on p53's effects on mitochondrial enzyme levels suggested p53's differential roles in pre-TCA vs. TCA metabolism. The approach was further applied to a p53-activating anticancer agent nutlin, which revealed its effects that are not expected from its p53 level regulation. Our approach can be easily applied to other systems where metabolism needs to be investigated in an organelle-specific manner.

**Poster #: 250**

**Abstract #: 2152**

**Abstract Title:** Dynamic LC retention times prediction for marker candidate's identification: steroidomics as a case study.

**Authors:** Serge Rudaz, Giuseppe Marco Randazzo, Julien Boccard, Fabienne Jeanneret, David Tonoli, Davy Guillarme, Alessandra Nurisso, Laura Goracci, Stephanie Hambye,

**Presenting Author Affiliation:** University of Geneva

**Abstract Submission:**

Due to the numerous pathologies related to potentially altering steroidogenesis (infertility, cancer, diabetes, obesity, etc.), this study aimed to assess the capabilities of UHPLC-HRMS for the simultaneous untargeted detection (metabolomics) and quantification of key steroids (steroidomics) in biological matrices. For the latter, a chemically-driven feature selection with database matching (HMDB, LipidMaps) was performed to extract data acquired in both positive and negative mode. This strategy constitutes a potent approach to screen and classify potential or confirmed toxicant affecting adrenal steroidogenesis based on the H295R cell model. The data mining strategy (Consensus O-PLS) was demonstrated to be a relevant approach to extract and highlight marker candidates. However, as often in "omics"-like strategies, even with the information of accurate mass, the definitive assignment of identity remains the main bottleneck. As example, an unknown steroid detected with an accurate mass of 305.2038 suggests a molecular formula of  $C_{19}H_{28}O_3$ , and nineteen (19) possible isomers could correspond. Without informative MS/MS fragmentation spectra due to the absence of characteristic diagnostic ions, the prediction of chromatographic retention times remains the decisive tool for identification. Models based on the chromatographic linear solvent strength (LSS) theory were used to predict retention times under any gradient conditions through two variables:  $\log k_w$  and  $S$ .  $\log k_w$  represents the extrapolated value of the retention factor  $k$  in pure water,  $S$  represents a constant molecular parameter for a given compound and fixed experimental conditions (i.e., the stationary phase chemistry). Using an iterative procedure, which extrapolates  $\log k_w$  and  $S$  from each compound from two gradient retention times, the prediction of retention values is not limited to an instrument or a specific gradient condition. Taking into account the analytical conditions used the retention time prediction the number of putative compounds was decreased and successfully identified in the cellular culture.

**Poster #: 251**

**Abstract #: 2250**

**Abstract Title:** Avoiding compound miss-annotation caused by MS in-source decay using data-independent acquisition

**Authors:** yasumune nakayama, Takeshi Bamba, Eiichiro Fukusaki,

**Presenting Author Affiliation:** sojo university

**Abstract Submission:**

Non-target metabolomics is emerging technology to search biomarkers, novel compounds, and so on. Recent advancement of mass spectrometry (MS) dramatically expand the possibility of the non-target metabolomics. Advancement of MS spec, tandem MS (MS/MS) technology, and information technology improved the annotation system of unknown peaks. However, the problems of miss annotation following to in-source decay is still remains. Labile ions are sometimes fragmented in ion source due to the high voltage or temperature. Difficult point is that these fragments are sometimes homologous to the ions of other compounds. Meaning, these fragments are miss annotated automatically by software applications even though high resolution MS and product ion scan are used. Here we introduce the new approach to solve the problem using data-independent acquisition (DIA) of liquid chromatography (LC) coupled with MS/MS. Standard reagents and yeast cell extracts were introduced to Nexcera system (Shimadzu, Kyoto, Japan) and analyzed with TritpeTOF 5600+ System (AB SCIEX, MA, USA). Data was acquired by sequential window acquisition of all theoretical mass spectra (SWATH) acquisition or information dependent acquisition (IDA). Miss-annotation were computationally detected using several properties of peaks. Firstly, as a reference, we constructed the library of in-source decay fragments from standard reagents using IDA. For several analytes, misleading fragments were observed. And our approach successfully detected the miss annotation caused by these fragments by analysis of SWATH data. These results indicate the possibility of our new approach to improve the non-target metabolomics.

**Poster #: 252**

**Abstract #: 2462**

**Abstract Title:** Developing sample-specific workflows for broad and sensitive metabolomics

**Authors:** Prasad Phapale, Theodore Alexandrov, Maria Elena Diaz-Rubio, Andrew Palmer, Dominik Fay, Ivan Protsyuk,

**Presenting Author Affiliation:** EMBL

**Abstract Submission:**

Metabolomics is advancing across a wide spectrum of biological samples from complex animal models to single cells. Basic biological research deals with diverse samples ranging from biofluids, cultured cells, organoids to animal, insect or plant models. The variety of this samples sources poses considerable metabolomic challenges in terms of sample amount, metabolite separation, quantification and availability data analysis resources. Often routine sample preparation and LC-MS protocols are not directly transferable to such samples to obtain required metabolome coverage and sensitivity with confident identifications. We present here our sample-specific LC-MS workflows and propose an approach to achieve broader and sensitive metabolomic profiles across different sample types.

Apart from developing sample extraction, enrichment, and LC-MS protocols, considerable efforts were put into developing open-source data analysis tools (<https://github.com/alexandrovteam/Optimus>, see the abstract by Ivan Protsyuk) and creating well-curated open-access EMBL MCF LC-Orbitrap-MS/MS spectral library of over 700 authentic metabolite standards. The library is already integrated into GNPS and is publicly available through our interactive web-based service Curatr (<http://www.embl.de/mcf/curatr>, see the abstract by Maria-Elena Diaz-Rubio).

Our LC-MS based sample and class specific workflow consists of novel features: 1) SPE-based sample enrichment for improving metabolite class-specific sensitivity, 2)

modified chromatographic methods for better separation and improve ESI-MS ionization to achieve broader metabolome coverage, 3) Using our EMBL MCF library that includes: a) RT index to address issue of LC-MS RT variability, b) MS response factor calculations for better quantification, c) confident and automated metabolite identification using in-house MS/MS spectral library along with in silico predictions. Also, during LC-MS analysis metabolites in biological matrix behave differently than standards. Hence, we have generated matrix-specific LC-MS/MS libraries for reliable identifications.

Our multi-faceted efforts enabled us to provide services across a wide range of biological questions, including knockout experiments in bacteria, zebrafish embryos, spatial structures in tumor organoids (<http://www.embl.de/mcf>).

**Poster #: 253**

**Abstract #: 2531**

**Abstract Title:** From 'peakomics' to metabolomics in LC-MS global profiling of human plasma

**Authors:** Miriam Navarro, Oriol Senan, Xavier Domingo-Almenara, Jordi Capellades, Antoni Aguilar-Mogas, Jesus Brezmes, Marta Sales-Pardo, Roger Guimera, Oscar Yanes,

**Presenting Author Affiliation:** CIBERDEM

**Abstract Submission:**

Most efforts to survey the metabolome in global untargeted studies have focused on improving biological extraction, chromatography methods and MS conditions to increase the number of spectral ion features. However, very little attention has been paid to the robust annotation of isotopes, adducts and in-source fragments through spectral deconvolution. In this work, we analyze human plasma from different extractions and LC/MS conditions that include ESI and APCI in both positive and negative ionization mode, to systematically resolve and annotate MS spectra using two novel computational approaches called AddClique and eRah. For the first time, we calculate and partially validate the real number of metabolites, the distribution of ion features in isotopes, type of adducts and in-source fragments, which has enabled us to estimate the actual number of known and unknown metabolites detected by LC/MS in human plasma.

**Poster #: 254**

**Abstract #: 2204**

**Abstract Title:** IMPROVING CONFIDENCE IN METABOLITE IDENTIFICATION IN NON-TARGETED LC-HRMS METABOLOMICS

**Authors:** Hector Gallart-Ayala, Shama Naz, Stacey Reinke, Caroline Mathon, Richard Blankley, Craig E. Wheelock,

**Presenting Author Affiliation:** Karolinska Institutet

**Abstract Submission:**

Non-targeted mass spectrometry-based metabolomics continues to be the gold standard for metabolic screening. Accurate metabolite identification and integration remain major barriers in this approach, but are essential for translating the resulting analytical data into meaningful biological results. We propose a novel approach for overcoming these obstacles that is based on extensive metabolite characterization and combines full scan and all ion fragmentation (AIF) MS/MS acquisition.

Using the Agilent PCDL Manager, we have created a database for ~700 metabolites that were rationally selected based upon their known importance in biochemistry and inflammatory disease. For each metabolite, experimental MS and MS/MS characterization in positive and/or negative electrospray was performed. The MS/MS experiments were conducted by fragmenting the most abundant precursor ions observed in the MS spectrum at four collision energies: 10, 20, 30 and 40eV. This custom library contains extensive experimental information, including: i) full scan MS, ii) MS/MS spectra of the most abundant precursor/adduct ions (e.g.,  $[M+H]^+$ ,  $[M+Na]^+$ ,  $[M-H]^-$ ,  $[M-HCOO]^-$ ), and iii) the retention time (RT) in two orthogonal chromatographic separation systems: reversed-phase and HILIC.

This exhaustive metabolite characterization has been used to build a metabolite screening method, which we have applied to multiple biological samples including plasma, serum, urine and cell supernatant. Metabolite identification was based on combining accurate mass/retention time (AMRT) with tandem mass spectrometry information provided by the all ion fragmentation (AIF) acquisition mode. Furthermore, the use of an ion ratio calculation (precursor ion/product ion) has been included to increase the specificity of metabolite identification and subsequent peak integration. This strategy can assist in resolving co-eluting isobaric compounds at the MS level by selecting a product ion as quantifier ion instead of the precursor ion. The utility of this method for molecular phenotyping has been demonstrated via the application to multiple clinical cohorts of individuals with asthma.



**Poster #: 255**

**Abstract #: 2105**

**Abstract Title:** MetFamily – a novel tool to coin data into information

**Authors:** Hendrik Treutler,

**Presenting Author Affiliation:** Leibniz Institute of Plant Biochemistry

**Abstract Submission:**

Using untargeted approaches, modern UPLC-QTOF instrumentation is capable of generating thousands of exact mass to retention time features from the MS1 and the MS/MS level. Despite big instrumental efforts the number of structure-identified features typically remains very low and structure elucidation of all features from a biological sample set is unfeasible.

In order to reduce this discrepancy, we developed the freely available web application 'MetFamily' (accessible via <http://msbi.ipb-halle.de/MetFamily/>). In the proposed workflow which is fully untargeted, we import metabolite profiles of several samples and a deconvoluted spectra library representing thousands of measured MS/MS spectra from MS-DIAL (Tsugawa et al., 2015). After alignment and binning of MS/MS features (fragment ions and neutral losses) from individual compound spectra, a new data matrix structure is generated. This new matrix structure combines the MS1 abundances of all samples and the binned MS/MS information in a single spreadsheet. This type of data re-organisation enables two orthogonal analyses on this data set. Using principal component analysis (PCA), we detect group-discriminating MS1 features based on MS1 abundances. By contrast, using hierarchical cluster analysis (HCA), we uncover various clusters of MS1 features based on similarities in the MS/MS spectra. Within HCA similarity clades, MetFamily identifies prevalent MS/MS features that are characteristic of distinct metabolite families and allows for their annotation. Since 'MetFamily' dynamically links PCA and HCA, the analysis can be constrained to group-specific MS1 features as well, highlighting up- or downregulated metabolite families in a biological context. Moreover, it is possible to select sets of MS1 features from structure-indicative fragment ions, neutral losses, or combination thereof. This function should greatly advance the selection of metabolite groups in biological or genomic studies.

Tsugawa, H. et al. Nature methods (2015) 12, 523-526.

**Poster #: 256**

**Abstract #: 2537**

**Abstract Title:** Identification and quantitation of 2',3'-cyclic nucleotides in murine tissues

**Authors:** Heike Bähre, Roland Seifert, Volkhard Kaefer,

**Presenting Author Affiliation:** Institute of Pharmacology, Hannover Medical School

**Abstract Submission:**

Nucleoside 3',5' cyclic monophosphates (3',5'-cNMPs) like 3',5'-cyclic adenosine (3',5'-cAMP) and 3',5'-cyclic guanosine (3',5'-cGMP) are well known second messengers which are involved in various biological processes. In the past few years it becomes obvious that in addition to 3',5'-cAMP and 3',5'-cGMP their 2',3'-analogues do occur in eukaryotic systems [1,2,3]. These studies mainly focused on 2',3'-cAMP but only little is known about the source and the tissue distribution of other 2',3'-cNMPs. 2',3'-cGMP can be found in significant amounts in rabbit kidney and pancreas [1]. Furthermore 2',3'-cAMP, 2',3'-cGMP, 2',3'-cCMP and 2',3'-cUMP were detected in various rat tissues [4].

The occurrence 2',3'-cAMP, 2',3'-cGMP and 2',3'-cCMP is consistent with the hypothesis that at least 2',3'-cAMP is a degradation product of mRNAs [5]. Therefore 2',3'-cUMP should be detectable as well. For this reason, we developed and validated a sensitive and specific liquid chromatography-coupled mass spectrometry (LC-MS/MS) method for identification and quantitation 2',3'-cNMPs simultaneously with 3',5'-cNMPs (cAMP, cCMP, cGMP, cUMP) [6]. Using this method we systematically analyzed mouse tissues (brain, thymus, heart, lung, liver, pancreas, spleen, kidney, bladder, testis, and the female reproductive system) for the presence of 2',3'- and 3',5'-cNMPs, respectively.

Besides 2',3'-cAMP and 2',3'-cGMP, remarkably high levels of 2',3'-cCMP and 2',3'-cUMP were detected in murine heart, kidney, spleen, liver, pancreas and lung.

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**Poster #: 257**

**Abstract #: 2601**

**Abstract Title:** Identification of LC-MS metabolite fragments using IROA (Isotopic Ratio Outlier Analysis)

**Authors:** Felice de Jong, Chris Beecher,

**Presenting Author Affiliation:** IROA Technologies, LLC

**Abstract Submission:**

The standard workflow for unbiased metabolomics matches peaks against a library for identification and finds a large number of unknowns since fragments are rarely identified as such. Source variations yield fragmentation variation therefore, spectral databases are of little use in the face of instrumentation variation, and data impurities stemming from artifacts, noise and ion-suppression. We have developed a general method for associating all fragments, adducts, dimers, etc., that also makes the structure elucidation of unknowns easier.

The IROA protocol incorporates stable-isotopes into metabolites, creating stable-isotopic Internal Standards (IS) for each and every metabolite measured so that specific alterations can be accurately measured and quantitated. Biochemically-complex IS are generated using growth media wherein all natural abundance  $^{12}\text{C}$  compounds (amino acids, sugars etc.) are replaced with randomly and universally 95%  $^{13}\text{C}$ -labelled compounds (95%  $^{13}\text{C}$  media) so when populations of cells are incubated in such media, all biological components in the cells, including metabolites, carry unique  $^{13}\text{C}$  signatures. When an IS, so created, is added to natural abundance samples and analyzed by MS, each metabolite peak carries a ready identifier of its origin, an enhanced M-1, M-2 etc. for the 95% $^{13}\text{C}$  IS and natural abundance M+1 for the  $^{12}\text{C}$  (Experimental) sample. Because of the presence of the IS all adducts and fragments may now be correctly identified even within areas of high co-elution, by the characteristics of the IROA peaks without the need of a fragmentation library.

ClusterFinder software identifies IS parent ions and their collective fragments, adducts, and provides structural confirmation of metabolites since the masses and ratios between the IS and its NA analogues will be a unique determinant for each such cluster. Furthermore, for each fragment the number of carbons present and their monoisotopic masses provide accurate formulae which supports structure elucidation of the parent compound in an unknown.

**Poster #: 258**

**Abstract #: 2606**

**Abstract Title:**  $^{13}\text{C}$ -labeled dimethyl- $\alpha$ -ketoglutarate as a new tracer for real time metabolomic monitoring of TCA cycle in live cells.

**Authors:** Yong Jin An,

**Presenting Author Affiliation:** Seoul National University

**Abstract Submission:**

Recent advances in metabolic research have been made, at least in part, through  $^{13}\text{C}$ -labeled tracers that can give flux information on metabolic pathways. Among tracers,  $^{13}\text{C}$ -glucose and  $^{13}\text{C}$ -glutamine are the most commonly used, because they can be easily transported across cell membrane and serve as precursors for a variety of metabolites. As tracers show different labeling efficiency on a given pathway, it is important to select an appropriate tracer for a particular purpose. One of the most fundamental metabolic pathways in eukaryotic cells is tricarboxylic acid (TCA) cycle. Recent evidence suggests that TCA cycles are essentially involved in cancers, especially for cancer stem cells and circulating cancer cells, and much interest has been raised in measuring the flux through TCA cycle. However, conventional  $^{13}\text{C}$ -tracers for TCA cycle flux measurement are not direct TCA intermediates, and therefore, the measurement can be affected by modulation of preceding metabolic steps. In addition, specific tracers for real-time measurement of TCA cycle are not available.

In this study, dimethyl  $^{13}\text{C}$ - $\alpha$ KG was synthesized as a new tracer and applied to different types of living cells to assess TCA activities in real-time with heteronuclear NMR.

**Poster #: 259**

**Abstract #: 2065**

**Abstract Title:** Retention time indexing in RP-LC-MS based metabolomics for enhancing metabolite identification: A cross-lab trial

**Authors:** Michael Witting, Nina Sillner, Dany Spaggiari, Serge Rudaz, Jutta Lintelmann, Jörg-Peter Schnitzler, Olga Begou, Georgios Theodoridis, Helen Gika, Philippe Schmitt-Kopplin, Michael Quilliam,

**Presenting Author Affiliation:** Helmholtz Zentrum München

**Abstract Submission:**

Metabolite identification is still the major bottleneck in non-targeted metabolomics. Different levels of identifications have been proposed by the Metabolomics Society Identification Task group (Sumner et al 2007, plus paper with refined levels). The highest level of identification can be only achieved by comparison with an authentic standard using two independent properties such as retention times and MS/MS spectra. Having reference standards for all possible metabolites in a single laboratory is nearly impossible and not feasible. Several public repositories storing MS/MS spectra have been created, also covering various instrumentations including MS analyzers (e.g., Q-ToF, IT or ICR) (MassBank, Metlin etc.).

MS/MS spectra represent only one part of an accurate identification and can be ambiguous in case of isomeric compounds, wrong collision energy queries, etc. Retention times could provide valuable information in LC-MS based metabolomics but are comparable only over a certain range. Even when using the same column and mobile phase chemistries and column dimensions, retention times can vary between labs due to different LC instrumentation. The concept of retention time indexing (RTI), already widely used in GC-MS, can help simplify the process by converting retention times to the dimensionless retention index, which is only dependent on stationary phase chemistry and mobile phase composition.

Here we present our first preliminary results from a ring-trial using a novel RTI system based on a homologous series of substances purposely designed for LC-MS and several metabolite standards measured in 5 different laboratories using different LC-MS systems.

**Poster #: 260**

**Abstract #: 2487**

**Abstract Title:** Open LC-Orbitrap-MS/MS spectral library facilitated by Curatr, a webapp for library curation, browsing and sharing

**Authors:** M. Elena Diaz-Rubio, Prasad Phapale, Andrew Palmer, Dominik Fay, Theodore Alexandrov,

**Presenting Author Affiliation:** EMBL

**Abstract Submission:**

Metabolite identification using LC-MS/MS requires comparison of experimental measurements against a spectra library, carefully curated and annotated data collected from authentic standards on an identical analytical platform. Due to a high cost of authentic standards and time-consuming and complicated process of curation, a spectral library is a high-value resource for every LC-MS/MS metabolomics lab working with MS/MS data.

The popularity of Orbitrap mass analyzers in metabolomics is growing. However, no open LC-Orbitrap-MS/MS spectral library is currently available. We present and share our LC-Orbitrap-MS/MS EMBL MCF spectral library of over 700 authentic metabolite standards which covers major biochemical pathways and classes. The library was created as the part of setting up the EMBL Metabolomics Core Facility (see the abstract by Prasad Phapale). Every spectrum was manually curated considering LC elution profiles, accuracy of precursor ions (< 10 ppm) and interpretable fragmentation pattern. The EMBL MCF library is already integrated into GNPS to contribute to the vibrant natural products community.

We also present our novel open-source webapp Curatr (<https://github.com/alexandrovteam/curatr>) for creating, browsing, and sharing a spectral library. Curatr provides the capability to: 1) provide records on the standards in the lab along with chemical information on each standard, 2) browse curated MS/MS spectra alongside information on standard provenance, experimental conditions, adducts detected, and data origin, 3) facilitate selection of the best MS/MS spectra from across the elution profile of an authentic standard, aided by measures of quality.

We provide access to the EMBL MCF library through the Curatr webapp at <http://www.embl.de/mcf/curatr>. A spectral library from Curatr can be exported in multiple formats (including mzXML, mzML, MGF, MSP, CSV, and MetaboLights JSON) to be either used in a metabolite identification software or contributed to public repositories.

**Poster #: 261**

**Abstract #: 2542**

**Abstract Title:** Biological inference of metabolite identity from isotope labelled experiments.

**Authors:** Gavin Blackburn, Fiona Achcar, Karl Burgess,

**Presenting Author Affiliation:** Glasgow Polyomics, University of Glasgow

**Abstract Submission:**

Identification of metabolites from liquid chromatography - mass spectrometry (LCMS) data can be a long and laborious task. Isomeric compounds are hugely abundant in almost all biological data sets and determining which are present can include multiple follow up experiments and require changes in LC methods, addition of MS fragmentation and even purification of individual compounds so techniques that give more accurate structural information, such as NMR can be used. Currently in LCMS methods, the gold standard for identification in LCMS is to match retention time and fragmentation pattern to an authentic standard and show that other possible isomers do not match. Standards can be costly or unavailable and fragmentation cannot always resolve the analysis to a single isomer.

Isotope labelling studies have been employed to follow specific metabolic pathways in organisms, in a focused, non-metabolomics way. This allows for a much deeper understanding of how specific metabolites are being acted upon when biological systems are changed. It also adds confidence to the selection of specific isomers when putative identifications are being made. If a likely identification can be connected to the initial, labelled substrate through the metabolic pathways observed in the system, this adds weight to this identification being correct compared to other isomers whose formation cannot be explained based on the systems biology.

This work shows how the appearance of specific labelling patterns in metabolites from a range of biological systems, both with and without authentic standards, can be used to intelligently select one putative identification over another and help build up a picture of the biology of the system in a manner that allows for easier interpretation of the data. We have shown this in a range of pathways, such as the production of malate independently of the TCA cycle.

**Poster #: 262**

**Abstract #: 2382**

**Abstract Title:** UPLC-QTOF-MS based serum metabolomic effect of diabetic kidney disease in Chinese patients

**Authors:** Tung Ting Sham, Chi On Chan, Na Ge, Hui Li Sun, Daniel Kam Wah Mok,

**Presenting Author Affiliation:** Department of Applied Biology & Chemical Technolog

**Abstract Submission:**

Diabetic nephropathy is a common serious complication of diabetes mellitus and one of the major public health problems worldwide with a prediction of more than half a billion suffers by 2030. This study aimed to discover serum metabolites that predict the progress of renal failure. Serum samples of total 36 inpatients with type 2 diabetes, three groups with diabetic kidney diseases at different stages according to the clinical test of glomerular filtration rate and nine normal individuals (control group) were collected from the Shenzhen Traditional Chinese Medicine Hospital (Shenzhen, China). A non-target metabolomic protocol with UPLC-QTOF-MS was applied. After data processing and elimination of unstable metabolites (normalized abundance in the quality control samples >30% of relative standard derivation) by Progenesis Q1 metabolomic platform (Waters), 685 and 1211 metabolites were detected in negative and positive ionization modes. PLS-DA showed a change of metabolic profiling between the four groups. Among them, phenylalanine and butyrylcarnitine levels were upregulated from normal, patients of diabetes to that of diabetic nephropathy whereas a growing trend of lysophosphatidylcholines (16:0, 18:0, 18:1, 18:2), lysophosphatidylethanolamine 18:0 were observed from the diabetic patients to those with kidney dysfunction. A more distinct separation was found between the group at the end stage of diabetic nephropathy and those at the first two stages and diabetic patients. At the end stage, markedly elevated serum concentration of phenylacetylglutamine, phenylalanine, cresol sulfate, indoxyl sulfate, butyrylcarnitine, were associated with the deteriorated glomerular filtration while the bilirubin and hydroxyvitamin D3 levels were also significantly depleted ( $p < 0.05$ ), which suggested that the diabetic patients with the observation of such transition could be greatly along with a deterioration of kidney clearance function. These results would be confirmed in a large subsequent prospective cohort.



**Poster #: 263**

**Abstract #: 2348**

**Abstract Title:** Exploring the fragmentome with unsupervised clustering and topic models

**Authors:** Justin Van Der Hooft, Joe Wandy, Sandosh Padmanabhan, Ronan Daly, Michael Barrett, Karl Burgess, Simon Rogers,

**Presenting Author Affiliation:** Glasgow Polyomics, University of Glasgow

**Abstract Submission:**

Untargeted metabolomics studies have the potential to answer important questions in many areas of the life sciences. Unfortunately, this potential is not fully realized since currently available tools are not able to extract the key biochemically relevant information from vast amounts of metabolomics data in an unsupervised and comprehensive manner. Here we use computational tools based on clustering and topic modelling to create molecular networks of biological extracts based on data-dependent MS/MS gas-phase fragmentation experiments.

One of the classical methods for judging spectral similarity is cosine scoring, which uses all product ions and their intensities to create vectorised spectra that are then compared. Here, we will demonstrate the use of molecular networking based on cosine similarities applied to urine metabolomics data to screen for antihypertensive drugs and their metabolites. We are able to successfully identify 13 drug related clusters. Furthermore, endogenous metabolites also formed clusters of carnitine derivatives, and conjugates containing glutamine and glutamate.

Molecular substructures often result in a few characteristic fragments or losses that get diluted in the full MS2 spectrum and can be easily missed by classical spectral similarity. To overcome this problem we also demonstrate 'MS2LDA', a topic modelling approach inspired by text-mining algorithms. MS2LDA extracts biochemically-relevant molecular substructures ('Mass2Motifs') from a collection of fragmentation spectra as sets of co-occurring molecular fragments and neutral losses. Metabolites can then be grouped according to shared substructures. We will show how it effectively establishes biochemical relationships of urine metabolites based on substructures including drug and endogenous metabolite (i.e., amino acid and nucleotide) related Mass2Motifs.

We conclude that building networks via cosine similarity and MS2LDA provide novel, complementary insights through their ability to pool data across the entire set of generated MS2 spectra within an analysis rather than restricting fragmentation data to one-to-one comparisons with spectral databases.

**Poster #: 264**

**Abstract #: 2031**

**Abstract Title:** Optimized UPLC-MS for High Precision Large Scale Metabolic Phenotyping of Human Urine

**Authors:** Matthew Lewis, Jake Pearce, Konstantina Spagou, Simon Lovestone, Paul Elliott, Zoltan Takats, Elaine Holmes, Jeremy Nicholson,

**Presenting Author Affiliation:** MRC-NIHR Phenome Centre

**Abstract Submission:**

Hyphenated ultra-performance liquid chromatography and mass spectrometry (UPLC-MS) is a fundamental tool in the pursuit of metabolic phenotyping and has been applied to studies of increasing size in an effort to better understand the molecular mechanisms underpinning phenotypic variation within human populations. While the technique is mature, the seemingly inevitable need to analyse large studies in multiple analytical batches poses a challenge for researchers and impacts the quality of data produced. Reducing the need for analytical batches and consequentially the need for data correction procedures requires a holistically optimized platform capable of sustained analysis on an industrial scale. Our efforts at shaping such a platform are described herein, utilizing complimentary chromatographic techniques with high resolution time-of-flight (ToF) mass spectrometry to generate high precision results for studies of 1000 samples or more. Applications to the study of human urine are discussed, highlighting the success of this work in terms of laboratory efficiency and data quality.

**Poster #: 265**

**Abstract #: 2100**

**Abstract Title:** Separation of isomers in lipidomics and metabolomics experiments by high resolution ion mobility-mass spectrometry

**Authors:** Michael Groessl,

**Presenting Author Affiliation:** TOFWERK

**Abstract Submission:**

Here we show for the first time the separation of a wide range of isomeric biomolecules using IMS at atmospheric pressure coupled to TOF MS. We demonstrate the separation of isomeric metabolites such as saccharides, glycans, sugar phosphates and amino acids. Additionally, the technique is shown to also very efficiently separate different isomeric lipids, comprising of regioisomers (different positions of acyl chains on the glycerol skeleton), double bond positional isomers and stereoisomers (cis/trans double bond geometry)

Data was obtained both by direct infusion IMS-MS as well as coupled to liquid chromatography (LC-IMS-MS). For the direct infusion measurement of complex samples such as metabolite or lipid extracts, an increase of detected features of over 40% by IMS-MS compared to MS only was observed. When running the instrument in LC-IMS-MS configuration, the additional IMS dimension significantly improves compound identification compared to LC-MS as collision cross sections can be used as unique identifiers in addition to accurate mass, retention time and fragmentation spectra.

Combined with multiplexing and post-processing techniques, ion mobility resolving power above 200 is routinely obtained. Multiplexing also increases ion transmission over 200 times and S/N ratios 10 times compared to conventional pulsed mode.

**Poster #: 266**

**Abstract #: 2247**

**Abstract Title:** A Targeted LC-MS/MS Method and Acquisition Database Optimized for Central Carbon Pathway Metabolites

**Authors:** Mark Sartain, Amy Caudy, Adam Rosebrock,

**Presenting Author Affiliation:** Agilent Technologies, Inc.

**Abstract Submission:**

A major challenge in metabolomics is achieving reproducible, robust chromatographic resolution with a single analytical LC/MS method for endogenous cellular metabolites due to their diversity of physiochemical properties. A more manageable chromatographic solution is to develop pathway-targeted methods. We developed a highly reproducible and robust ion-pair based reverse phase (IP-RP) chromatographic method, providing efficient separation of anionic and hydrophobic metabolites. This method enabled simultaneous analysis of >200 molecules representing many metabolite functional classes, including amino acids, citric acid cycle intermediates and other carboxylic acids, nucleobases, nucleosides, phosphosugars, and fatty acids. In conjunction with the IP-RP method, a triple quadrupole mass spectrometer operated in dynamic MRM (dMRM) mode enabled sensitive detection of analytes across a wide dynamic range. Compound MRM peak retention times were curated into a database browser format that allowed easy import into the LC/MS acquisition software. The curated retention times increased confidence in assigning the MRM signals to the correct metabolites in complex matrices and also enabled dMRM acquisition where mass spectrometer cycles are dedicated to a given compound in a window flanking its elution. This permitted efficient targeted detection enabling improved limits of detection and the measurement of more metabolites. Primary transitions, up to two per compound, were chosen based on fragment ion selectivity and signal-to-noise response. The database was curated with in-chromatogram optimized fragmentor and collision energies to provide sensitive instrument-optimized performance. In conjunction with the IP-RP method, the optimized assay enabled sensitive detection of analytes across a wide dynamic range. Furthermore, the targeted nature of the assay produced data implicitly associated with compound names and database identifiers enabling seamless downstream differential and pathway analysis. As proof of principle, we deployed this assay to measure relative changes in yeast central carbon pathway metabolites comparing media with acetate or glucose as the primary carbon source.

**Poster #: 267**

**Abstract #: 2249**

**Abstract Title:** High Performance Ion Pair-Reverse Phase LC/Q-TOF Method for Profiling Diverse Classes of Endogenous Metabolites with Separation of Important Isomers

**Authors:** Yuqin Dai,

**Presenting Author Affiliation:** Agilent Technologies

**Abstract Submission:**

High resolution accurate mass LC/TOF or Q-TOF MS is routinely used in metabolomics for discovery work. Some analytical challenges remain, such as retention of ionic metabolites, chromatographic separation of biologically important isomers, and detection of diverse classes of endogenous metabolites in a single analytical run.

To address these challenges, we have developed a robust ion pair-reverse phase (IP-RP) LC Q-TOF MS method using a C18 column with tributylamine as an ion pairing agent. The chromatographic gradient was optimized to achieve a baseline separation for several pairs of important isomers such as citrate/isocitrate and D-glucose-6-phosphate/alpha-D-glucose-1-phosphate, etc. Another important element of the optimization is the mobile phase pH. We found that slight changes to the pH of the mobile phase can alter the retention of sugar phosphates and nucleotide triphosphates, however, the signal intensities of a wide range of metabolite classes were impacted. For the three pH conditions (5, 5.8 and 7.5) evaluated here, the mobile phase at pH of 5.8 provides the overall best results in terms of the chromatographic separation for the isomers and acceptable sensitivity among the different classes of metabolites.

To further enhance signals of small, labile metabolites, we utilize an intelligent tuning algorithm to optimize the ion transmission of the Q-TOF system. We will demonstrate how this algorithm allows simplified customizable instrument optimization to meet metabolomics application needs.

This IP-RP LC/Q-TOF method clearly demonstrated superior analytical performance. It enables simultaneous detection of several pairs of biologically important isomers and a diverse set of endogenous metabolites including amino acids, carboxylic acids, sugars and sugar phosphates, nucleotides and Coenzyme A derivatives. This reduces the need for multiple LC/MS methods. An application of this method for analysis of real world biological samples is being further studied.

**Poster #: 268**

**Abstract #: 2296**

**Abstract Title:** Development of a chemical derivatization - UPLC-MRM/MS method for quantitation of bile acids in dried blood spots

**Authors:** Jun Han, Georgia Mitsa, Karen Lin, Juncong Yang, Christoph H. Borchers,

**Presenting Author Affiliation:** UVic-Genome BC Proteomics Centre

**Abstract Submission:**

Quantitation of bile acids (BAs) in biological samples is currently dominated by UPLC-multiple-reaction monitoring (MRM)/MS. Due to the lack of low-energy collision-induced fragment ions, or due to the low analytical sensitivities of MRM, for detection of unconjugated BAs on triple-quadrupole instruments, reliable measurements of many unconjugated BAs in biological samples are often compromised. To ameliorate this situation, we describe herein a chemical derivatization – UPLC-MRM/MS method for precise and accurate quantitation of more than 30 BAs in dried blood spot (DBS).

3-Nitrophenylhydrazine (3-NPH) was used as an efficient pre-analytical reagent to completely derivatize 28 unconjugated and glycine-conjugated BAs under an optimized reaction condition (50 °C for 45 min). The derivatives showed good in-solution stability at 5 °C for 96 hours without significant degradation. The derivatization had no effect on taurine-conjugated BAs, which made it practical to apply the method to simultaneously quantitate the unconjugated and glycine-conjugated BAs as well as 10 taurine-conjugated BAs in single set of UPLC- negative-ion ESI-MRM/MS runs. Good separation of all the analytes was achieved on a reversed-phase C-18 column. This developed method was validated for its analytical sensitivity and the precision and accuracy of quantitation. Application of this method enabled successful quantitation of 31 detectable BAs in DBS and the measured concentrations displayed excellent correlation ( $R^2=0.995$ ) between DBS and blood plasma. Analysis of the BAs in DBS stored at different temperatures for different time periods indicated good chemical stability of the measured BAs in the spots stored at 4 °C or at a lower temperature. Taurine-conjugated BAs in the spots stored at room temperature showed significant degradation. In summary, a new UPLC-MRM/MS method that incorporates chemical derivatization for determination of BAs in DBS has been developed and this method overcomes a major technical disadvantage of MRM/MS for the analysis of unconjugated BAs.

**Poster #: 269**

**Abstract #: 2124**

**Abstract Title:** Evaluation of High Speed, High Resolution Data Independent Acquisition for the Analysis of Metabolomic Flux, Kinetics and Pathway Mapping

**Authors:** Stephen Ayris,

**Presenting Author Affiliation:** SCIEX

**Abstract Submission:**

Scientists are looking at metabolomics to answer challenging questions and complement existing datasets. LCMS techniques have greatly increased the quality of metabolomics datasets, however, most of this work has focused on the identification of metabolites versus the quantitation of metabolites and pathways. Most quantitative studies have focused on the measurement of steady-state metabolite levels where experimental variability can mask the true degree of metabolic regulation. Data independent technique applied to the measurement of the flux heavy isotopes in a metabolic pathway shows great potential to elucidate the regulation / kinetics of metabolic pathways. Herein we evaluate the merits of various high resolution approaches to the measurement of metabolic flux.

CHO cells were cultured under sterile conditions and split into replicate flasks. Several control and treatment flasks were created. Controls were created by feeding normal media as well as media prepared with stable isotope labeled  $^{13}\text{C}_6$ -glucose and  $^{13}\text{C}_5$ -Glutamate. One set of treatment flasks was treated with Hydrogen peroxide and 2-deoxy glucose to perturb the system. Cell supernatant was injected onto weak anion exchange chromatography coupled to an ESI QqTOF style mass analyzer. Acquisition was performed in positive and negative mode and was generated using a targeted, data dependent and data independent techniques. Identification of metabolites was performed using XCMSplus and metlin-database.

This work demonstrates the practicality of combining high levels of resolution and scan speed at the MS and MS/MS level. This level of performance can be leveraged to generate information rich targeted and data independent datasets for metabolomics flux studies

**Poster #: 270**

**Abstract #: 2157**

**Abstract Title:** Development of anionic metabolome analytical platform using ion chromatography-mass spectrometry (IC-MS)

**Authors:** Akiyoshi Hirayama, Masaru Tomita, Tomoyoshi Soga,

**Presenting Author Affiliation:** Keio University

**Abstract Submission:**

There are several MS-based platforms frequently used in the field of metabolomics such as GC/MS, LC-MS and CE-MS. 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, in which a capillary ion chromatograph was coupled with a Q Exactive mass spectrometer (Thermo Scientific) and run in full mass scan mode.

Firstly, influence of sheath liquid conditions on IC-MS-based metabolome analysis were investigated, and IPA with 0.1% acetic acid was selected as an optimum solution. Under this optimized condition, 49 anionic metabolites, including organic acids, sugar phosphates, nucleotides and coenzymes, were successfully separated and detected with a the mass spectrometer. Acceptable method validation results were obtained related to reproducibility, linearity and sensitivity of the IC-MS method. Notably, the concentration detection limit of the tested compounds were between 1 and 10 nmol/L with only small volume consumption (0.4  $\mu$ L). Finally, we had optimized sample preparation protocol for both cultured cell and blood samples for IC-MS analysis.

Currently, we are applying this platform to the anionic metabolomic profiling for several cancer cell lines. In conclusion, the developed IC-MS platform could be a powerful new tool for anionic metabolome analysis.



**Poster #: 271**

**Abstract #: 2130**

**Abstract Title:** Mammalian Tissue Metabolomes: A Qualitative Comparison

**Authors:** Andrew Chetwynd, Riccardo Di Guida, Elliott Palmer, Giovanny Rodriguez-Blanco, AnnMarie Withanage, Warwick Dunn,

**Presenting Author Affiliation:** University of Birmingham

**Abstract Submission:**

**Introduction:** Many biological studies investigating metabolism in mammals employ easy to acquire samples including serum, plasma, urine and saliva. Although these biofluids are easy to collect and provide the most frequently applied sample types in large-scale human population studies they do not accurately represent dynamic metabolism in tissues. When investigating pathophysiological mechanisms of health, ageing and disease tissue samples represent the biological site in which important mechanisms are operating. A detailed comparison of the metabolomes of different mammalian tissues has not been performed previously to our knowledge.

**Objectives and Methods:** In this study we have investigated the metabolite composition of five tissues (heart, liver, lung, kidney and muscle) collected from sheep. Tissues from four animals were extracted applying a monophasic extraction protocol (water/methanol/chloroform) and analysed applying a C18 reversed phase UHPLC-MS non-targeted metabolomics approach. Following data preprocessing with XCMS, the data were analysed applying univariate and multivariate analysis methods.

**Results and conclusions:** As expected, the metabolomes of each tissue clustered separately from each other when investigated applying PCA with muscle and heart being closely associated and separately with liver, lung and kidney being associated. Distinct metabolic differences associated with hydrophilic and lipophilic metabolites were observed and will be discussed.

**Poster #: 272**

**Abstract #: 2244**

**Abstract Title:** A Quality Assurance Program for Metabolomics: Can NIST Help?

**Authors:** Katrice Lippa, Bruce Benner, Jr., Nik Blonder, Werickson Rocha, David Sheen, Yamil Simon, Dan Bearden,

**Presenting Author Affiliation:** National Institute of Standards and Technology

**Abstract Submission:**

The National Institute of Standards and Technology (NIST) is the national metrology institute (NMI) for the United States. NIST has three decades of experience in providing quality assurance programs (QAPs) to the micronutrients, dietary supplements, clinical diagnostics, health monitoring, and environmental contaminant measurement communities (<http://www.nist.gov/mml/csd/qaps.cfm>) in the US and around the world. Hundreds of national and international laboratories (academic, government, R&D, hospital, and diagnostic testing) have participated in one or more of these QAPs. The charge of these programs is to help participants harmonize and improve the comparability of their community's measurements through feedback reports and workshops, development of measurement procedures, and development of reference materials. Coordinating these programs helps NIST to monitor and support the emerging measurement needs of the participating communities. Participation in these programs has been shown to improve the reliability of measurement results over time.

NIST has recently engaged in pilot interlaboratory comparisons for lipidomics and metabolomics to promote measurement comparability in this rapidly advancing yet still emergent field. For the pilot metabolomics interlaboratory study, NIST evaluated a suite of pooled urine materials by NMR, LC-MS and GC-MS-based platforms as candidate reference or "harmonization" materials for sample processing, instrumental analysis and data analysis procedures. The goal is for these materials to be evaluated principally by the metabolomics community via interlaboratory comparisons and shared data evaluations via a QAP for Metabolomics (qMet). The qMet concept is to provide individual laboratories with timely and affordable reference materials together with up-to-date metabolomics data, relevant method information, meaningful univariate and multivariate performance metrics and overall measurement (i.e., consensus) results via a publically-available website that reflects the current 'quality' status of measurements in the metabolomics community.

**Poster #: 273**

**Abstract #: 2055**

**Abstract Title:** A triphasic single-step method for rapid, comprehensive and simultaneous extraction of lipids, metabolites and proteins from a single plant sample.

**Authors:** Mohamed Salem,

**Presenting Author Affiliation:** Max Planck Institute of Molecular Plant Physiology

**Abstract Submission:**

Optimized analysis of selected metabolites or classes of metabolites requires specific extraction procedures in combination with specific analytical instrumentation. However, the most efficient specific extraction protocols unfortunately often only cover a very limited number of compound classes. As a consequence, many samples are needed to reach a comprehensive metabolic coverage. In this method, we describe a validated extraction work flow covering primary and secondary metabolites but also lipids and proteins from the same plant sample to cover all major metabolic pathways. Additionally, the method can be used also for comprehensive analysis of reserve starch and plant cell wall composition. Using *Arabidopsis thaliana* seed as a reference tissue, lipids, primary and secondary metabolites together with proteins were analysed using hyphenated mass spectrometry-based techniques (GC- and LC-MS). We have successfully used this method to annotate more than 200 lipid compounds, covering most of the classes involved in lipid metabolism. Additionally, we annotate more than 70 polar and semi-polar compounds using LC-MS method covering most of phenylpropanoids and glucosinolates. Further we cover more than 90 compounds involved in central metabolism. Additionally, we routinely obtained about 2000 protein identifications from *Arabidopsis* seeds but also the polysaccharide composition of the cell wall and the crystalline cellulose content. Accordingly we believe that this method could be used, with minor adaptations, to analyse metabolites, lipids and proteins from most biological samples.

**Poster #: 274**

**Abstract #: 2190**

**Abstract Title:** Target metabolomics assay for absolute quantification of omega-3 and omega-6 oxylipins in human plasma

**Authors:** Mark Haid, Marina Rudisch, Caroline Muschet, Jerzy Adamski,

**Presenting Author Affiliation:** Helmholtz Zentrum Muenchen

**Abstract Submission:**

Inflammation processes are known to be initiated and maintained by arachidonic acid (AA) derived omega-6 fatty acids. The enzymatic (and non-enzymatic) oxidation of AA leads to the formation of pro-inflammatory lipid species like prostaglandins, prostacyclins, leukotrienes, and several other hydroxylated eicosanoids. Recent studies have shown that the resolution of the inflammatory status is mediated by derivatives of the omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), the resolvins, maresins, and protectins. These molecules are directly involved in pro-resolving actions like reduction of cytokine expression and leukocyte infiltration. However, the balance of the pro- and anti-inflammatory status has to be well orchestrated. Otherwise sepsis, rheumatoid arthritis, asthma, or other autoimmune diseases may be the consequence. Furthermore, it has been shown that inflammatory processes are also involved in the aetiology of type 2 diabetes, cardiovascular diseases and cancer. Thus, investigation of the interplay of the omega-3 and omega-6 oxylipins may allow insight into the pathogenesis of complex diseases and may reveal new biomarkers and drug targets.

In order to support such future analyses we developed a quantification method for oxylipins.

Despite the challenge of high chemical similarity, instability, and low plasma concentrations of the oxylipins, we were able to establish a targeted metabolomics LC-MS method that allows the high-throughput quantification of > 50 omega-3 and omega-6 polyunsaturated fatty acids in human plasma. The assay comprises precursors and intermediates of the most important pro- and anti-inflammatory oxylipins known so far. Metabolites are separated within a 15 minute gradient LC run and subsequent injection into a Sciex 5500 QTrap system. Absolute quantification is based on a scheduled MRM method and the use of isotopically labelled internal standards. The assay has been validated according to the FDA standards.

**Poster #: 275**

**Abstract #: 2149**

**Abstract Title:** Real-time measurement of chemical composition of exhaled human breath by ambient ultrahigh resolution mass spectrometry

**Authors:** Xue Li, Lei Huang, Zhen Zhou,

**Presenting Author Affiliation:** Jinan University

**Abstract Submission:**

Exhaled breath metabolome provides important complementary information to the whole human metabolome, and is promising for non-invasive cancer diagnosis, environmental exposure assessment, etc. In this study, real-time measurement of chemical composition of exhaled breath by using ambient ultrahigh resolution mass spectrometry (A-UHRMS) was demonstrated. Direct sampling followed by secondary electrospray ionization (SESI) was achieved by applying a commercial Nano-ESI source a hybrid quadrupole orbitrap mass spectrometer was used for accurate mass detection at 15,000, 30,000, 60,000 and 120,000 resolution under both positive and negative ion detection modes. Exhaled breath fingerprints of 4 subjects (3 males and female, 25–35 years old) were obtained. Detection of low-intensity features separated from neighboring peaks as well as identification of interested features were especially benefited by using the ultrahigh resolution mass spectrometry based strategy. Phenol, pyridine, nitrate, sulfate, phosphate, organic acids, aldehydes, ketones, etc were observed in the breath samples. Interestingly, the adduct ion of  $[C_2H_6SiO]_6$  with ammonium ion was found in the breath samples of one male subject while the protonated  $[C_2H_6SiO]_6$  was not, and this may suggest the possibility of detecting exhaled ammonia by using the present method. Furthermore, the chemical composition of exhaled breath samples was investigated considering the sources of endogenous metabolites, food and drinks, indoor air, etc, and the benefits and challenges of direct breath analysis for studying exhaled breath metabolome were discussed.

**Poster #: 276**

**Abstract #: 2265**

**Abstract Title:** Automated high-throughput profiling of human plasma NMR-metabolites

**Authors:** hanna eriksson röhnsich, Elisabeth Mullner, Jan Eriksson, Peter Agback, Ali Moazzami,

**Presenting Author Affiliation:** department of chemistry and biotechnology, swedish

**Abstract Submission:**

Nuclear Magnetic Resonance spectroscopy (NMR) and Mass Spectrometry (MS) are the two key platforms employed in metabolic profiling of bio fluids. Although metabolic profiles provided by NMR allow for absolute quantitation of several metabolites, the process is rendered with difficulties due to the inherent complexity of biological fluids. Metabolic profiling requires deconvolution of individual spectroscopic signals from a sum of signals originating from several metabolites, before the target metabolite can be quantified. High-throughput metabolic profiling is particularly cumbersome as commonly used manual signal integration procedures are time-consuming. Therefore, automated strategies for targeted metabolic profiling have been introduced. We have implemented a rapid and easy to use automated strategy for high-throughput quantitative analyses of more than 60 eligible metabolites confirmed to be present in human blood<sup>5</sup>. Ultrafiltration and NMR analyses of human plasma samples from 1342 Swedish male volunteers provided metabolic profiles for quantitative analyses. For the automated quantification procedure total signal intensities in preselected spectral regions were extracted using spectral binning and an in-house signal selection routine. Overlaps between signals from different metabolites in these regions were then simultaneously deconvoluted using spectroscopic information available from commercial databases and in-house references. Ultimately the deconvolution procedure allowed us to instantly estimate sample specific intensities for each target metabolite. The corresponding concentrations in the samples were obtained by calibration using the signal of an internal standard of known concentration. In addition, we executed a manual targeted profiling integrating the same spectral regions in a step-wise fashion in order to account for overlaps. In general, estimated concentrations for metabolites above limit of detection displayed close similarities between procedures. However, the rate of the automated quantification procedure was superior. This study demonstrates a new possibility to reveal plasma levels of plasma metabolites from the complex metabolic profiles provided by NMR, in a high-throughput manner.

**Poster #: 277**

**Abstract #: 2478**

**Abstract Title:** Computational chemistry in metabolomics: The design and application of novel fluorescent tags for the detection of fatty acids by HPLC

**Authors:** Oliver Jones, Michelle Spencer,

**Presenting Author Affiliation:** RMIT University

**Abstract Submission:**

Fatty acids are traditionally analysed as Fatty Acid Methyl Esters (FAMES) via GC-MS. While Lipidomics is increasingly popular, since most fats and lipids don't fluoresce under UV light most studies rely on detection via expensive mass spectrometry equipment. An alternative is to add a fluorophore to the fatty acid molecule, but which one to use? We hypothesised that computational chemistry might be able help predict the properties of a new fluorophore (5-(4-(diphenylamino)phenyl)thiophene-2-carbaldehyde) developed at RMIT. Ab initio calculations, using the Gaussian09 software package, were used to calculate the optimised geometry, orbital electron density distribution and band gap ( $E_g$ ) of the fluorophore, an isolated fatty acid (propanoic acid) and the fatty acid molecule tagged with the fluorophore. The LUMO orbital density distribution of the fluorophore was found to be primarily located on the phenyl thiophene region of the molecule while the HOMO was more evenly distributed over the molecule with a greater contribution on the two phenyl amine groups. When the fatty acid is tagged with the fluorophore, the distribution of the HOMO/LUMO orbital density remains little changed, with no contribution from either HOMO or LUMO on the fatty acid chain. The segregation of the HOMO and LUMO densities allows charge transfer within the molecule leading to its good fluorescent properties and is consistent with the tagged acid retaining the fluorescent properties of the tag. It was found that the tag could be attached to common fatty acids using  $\text{BF}_3$  and methanol in a similar manner to the method currently used for the derivatization of fatty acids to FAMES. The study shows that i) computational data can be used to focus experimental work and that ii) Such linkages could achieve useful results and significant time savings in the design of new analytical methods in metabolomics and related areas.

**Poster #: 278**

**Abstract #: 2513**

**Abstract Title:** Strategies for within and between batch data correction for large-scale LC-MS metabolomics data

**Authors:** Carl Brunius, Lin Shi, Rikard Landberg,

**Presenting Author Affiliation:** Swedish University of Agricultural Sciences

**Abstract Submission:**

Untargeted metabolomics has become an important tool in biological and medical research by providing detailed information about metabolites in an organism. LC-MS is frequently used due to broad coverage of metabolites, high sensitivity and simple sample preparation. However, due to unstable instrumental conditions, data generated from multiple batches is typically affected by measurement errors from drift in mass accuracy and retention times between samples both within and between batches. These errors reduce the repeatability and reproducibility of the analysis and may decrease the power to detect biological responses and obscure the interpretation of data. Development of techniques for data correction before statistical analysis are warranted. We have therefore developed algorithms to address and correct for within- and between-batch variability in processing multiple batch untargeted LC-MS metabolomics data to increase the quality of the data. This includes: i) A within-batch cluster-based correction to normalize signal intensity drift by allowing multiple drift patterns within-batch, thus providing a combination of modelling detail and power ii) Alignment and merging of features that are systematically misaligned between batches. This is achieved by aggregating feature presence/missingness on batch level and combining similar features orthogonally present between batches and iii) A decision strategy for feature-wise determination of between-batch normalization method, based on a heuristic that assesses the suitability of normalizing by reference sample intensity. Used on authentic data, within-batch correction provided a decrease in median QC feature CV from 20.5% to 15.1% and between-batch alignment gave the benefit of both picking 13% more features and deconvoluting 13% of features previously erroneously aligned. Although developed for LC-MS based metabolomics data, these methods are generic and can be applied to other data suffering from similar limitations.



**Poster #: 279**

**Abstract #: 2603**

**Abstract Title:** Effective, standardized metabolomics workflow applied to 3 studies of cell toxicity

**Authors:** Rick Reisdorph, Nichole Reisdorph, Scott Walmsley, Samantha Bokatzian, Kevin Quinn, Roger Powell,

**Presenting Author Affiliation:** University of Colorado, Skaggs School of Pharmacy

**Abstract Submission:**

We have developed a standardized metabolomics workflow to enable rapid discovery of mechanisms of cellular toxicity. Information gained from independent studies is currently being combined to discover general and specific responses to toxicity. HEK293 or SH-SY5Y cells were treated, in separate time and dose experiments, using 3 different cellular toxins as follows: 200 uM AICAR from 0-150 minutes, taxol 0-48 hours, and cholera toxin 0-10 ug/ml. Liquid-liquid extraction using MTBE or a simple methanol precipitation were performed following quenching. MTBE extraction resulted in aqueous and lipid fractions. Reverse-phase C18 or HILIC were used for lipid and aqueous fractions, respectively, followed by LC/MS analysis in both positive and negative mode. Our standardized informatics workflow included Mass Hunter to extract compounds. Mass Profiler Professional and R were used to perform quality control, visualizations and an unpaired t-test was performed ( $p < 0.05$ ) for treated vs controls at each time point with Benjamini Hochberg FDR multiple testing correction. Finally, a 1.5-fold change filter was applied. Pathways enrichment was performed using Kyoto Encyclopedia of Genes and Genomes (KEGG). Metabolites were annotated using public and in-house databases. Identities were confirmed with standards and/or MSn where possible. In general, our methods result in the detection of 700-1000 compounds in aqueous fractions and over 1,500 lipid molecules, depending on cell type. Using our informatics workflow, we obtain between 25-72% coverage of major metabolic pathways. Major pathways perturbed in all experiments include glycerophospholipids, pyrimidine metabolism, histidine metabolism, and sphingolipid metabolism. A disruption in ABC transporters was more specific to taxol, perturbation of amino acids was highly associated with cholera toxin treatment. While glycerophospholipids were disrupted in all treatments, PC and PE downregulation was specifically related to AMPK activation by AICAR. Overall, our robust methodology enables next generation data analysis and informatics to more fully explore cellular response to toxicity.

**Poster #: 280**

**Abstract #: 2367**

**Abstract Title:** Association between obesity and amino acids: a metabolomics approach

**Authors:** DIRCE MARCHIONI, Augusto Carioca, Josiane Steluti, Andreia Miranda, Aline Carvalho, Ismael Silva, Alexsandro Silva, Regina Fisberg,

**Presenting Author Affiliation:** SÃO PAULO UNIVERSITY

**Abstract Submission:**

Obesity has reached epidemic proportions in many countries around the world and is strongly linked to a number of chronic diseases. Some amino acids, such as three branched amino acids and glutamine, are associated with metabolic disorders. The interaction between obesity and amino acids is complex and not yet fully elucidated. The metabolomics can be a useful tool for solving some of these metabolic disorders. The aim was to investigate the association between amino acids and obesity. Methods. Data came from a population based cross-sectional survey in Sao Paulo among 169 adults. A trained research nurse measured body weight, height and waist circumference (WC) using a standardized protocol. BMI was calculated by dividing weight (kilograms) by the square of height (meters). Obesity was determined based on BMI = 30 kg/m<sup>2</sup>. The branch chain amino acids (BCAA) are the sum of the valine, leucine, and isoleucine. The glutaminolysis ratio (Gluta Ratio) is (alanina+ aspartate + glutamate)/glutamine. Quantification of the metabolites was performed by mass spectrometry of the type targeted. Multiple linear regression models were performed to verify the association between amino acids (BCAA and Gluta ratio) and BMI or WC, adjusted for age, sex, energy intake, race, smoking, physical activity. Results. The sample was comprised of 52% men, 60% adults and mostly of white race (57%). The BMI was associated with BCAA ( $\beta=8.57$  p<0.001) and Gluta Ratio ( $\beta=0.02$  p<0.001). The WC was associated with BCAA ( $\beta=3.92$  p<0.001) and Gluta Ratio ( $\beta=0.01$  p<0.001). The concentration of BCAA ( $\beta = 82.67$ , p<0.001) and Gluta ratio ( $\beta = 0.211$ , p<0.001) were higher in obese compared with non-obese. Conclusion. Obesity was associated with three branched amino acids and glutamine metabolism.

**Poster #: 281**

**Abstract #: 2476**

**Abstract Title:** Comprehensive and Quantitative Metabolomic Analysis Using Chemical Isotope Labeling LC-MS for Disease Biomarker Discovery

**Authors:** Nan Wang, Xiaoling Su, Jingjing Lu, Derrick Blackmore, Zaeem Siddiqi, Liang Li,

**Presenting Author Affiliation:** Department of Chemistry University of Alberta

**Abstract Submission:**

Our lab applies a 'divide-and-conquer' strategy and develops chemical isotope labeling (CIL) LC-MS techniques for large scale profiling of amine, phenol and carboxylic acid submetabolomes, enabling sensitive and quantitative measurement of thousands of metabolites in different biospecimen.

In this study, we first developed metabolite extraction and CIL protocols for human fecal specimen.  $^{12}\text{C}/^{13}\text{C}$ -dansyl chloride (DnsCl) was used to label amine/phenol metabolites and  $^{12}\text{C}/^{13}\text{C}$ -p-dimethylaminophenacyl bromide (DmPA) was used for carboxylic acids. On average,  $2507 \pm 77$  and  $2208 \pm 57$  peaks pairs were detected using DnsCl and DmPA labeling, respectively. In-depth study on the effects of fecal sample storage conditions on metabolome profiling was conducted using 560 samples stored in different ways including storage after collection on ice, at ambient temperature, at  $-20^\circ\text{C}$  and  $-80^\circ\text{C}$ . Quantitative analysis on the changes of essential groups of endogenous metabolites will be presented.

The developed techniques were applied to investigate the molecular mechanism of acute liver failure (ALF) using eight D-galactosamine-induced ALF pigs. Feces collected at four time points were well separated in OPLS-DA analysis. The heat map of hierarchical clustering suggested several groups of metabolites such as organic cofactors and dipeptides show significant changes in similar patterns. Pathway enrichment analysis of these metabolites was performed and will be presented.

Lastly the CIL LC-MS methods were utilized to assess the serum metabolome in patients with myasthenia gravis (MG). We compared the metabolome profiles of 49 MG patients and 50 healthy individuals. PLS-DA analysis showed a clear separation of the two groups ( $R^2 = 0.98$ ,  $Q^2 = 0.80$ ). The Receiver Operating Characteristic (ROC) analysis of 7 metabolites produced an Area Under the Curve (AUC) value of 0.859 (0.806-0.920 at the 95% confidence interval) with 91% specificity and 70% sensitivity. These results provide key insights into disease mechanisms and substantiate the probability of finding metabolic biomarkers specific to MG.

**Poster #: 282**

**Abstract #: 2074**

**Abstract Title:** Advances in Targeted Omics Quantitation Using a Novel Scanning Quadrupole DIA Method

**Authors:** Christopher Hughes, Lee Gethings, Johannes Vissers, Keith Richardson, Jason Wildgoose,

**Presenting Author Affiliation:** Waters Corporation

**Abstract Submission:**

An alternative DIA mode of operation developed for a tandem quadrupole/oa-time-of-flight (ToF) mass spectrometer was applied for targeted lipidomics experiments using transition extraction lists and compound library based approaches. The  $m/z$  isolation range of the quadrupole was continuously and repetitively scanned with MS data acquired using a high-resolution ToF acquisition system capable of delivering up to 2000 spectra/s. Alternate MS scan data comprise precursor and CID product ions. The quadrupole mass range and resolution were investigated to determine the optimal balance between sensitivity and specificity. The resulting 2D data,  $m/z$  (ToF) vs.  $m/z$  (quadrupole) were processed and quantified using Skyline open source informatics and visualised in OpenMS.

Control, diabetic and obese plasma samples of varying phenotype were analysed and differentially spiked with standards, acting as pseudo QCs, and quantitative lipid changes determined. It was found that quadrupole transmission windows of 5 - 10 and 20 - 30 Da provided optimum lipid identifications. Qualitative information from the same data sets was obtained by extracting lipid class information based on neutral loss or product ion extraction. Human plasma samples were treated with isopropanol and centrifuged to precipitate proteins. The lipid-containing layer was collected and diluted to adjust the water content prior to analysis. 2DMS data were collected for the two complementary sample types and differently expressed fatty acids, phosphatidylcholines, triglycerides and phosphatidylserines, and apolipoprotein peptides, respectively, across the three conditions of interest quantified. Quantitative and statistical analysis of the 2DMS DIA data was conducted with Skyline embedded tools, and visualisation/profiling in either OpenMS or DriftScope. The obtained results were in good agreement with previous discovery studies and the expected changes in relation to disease and/or phenotype.

**Poster #: 283****Abstract #:** 2095**Abstract Title:** How to carry out large-scale metabolomics studies based on GC-MS or LC-MS**Authors:** Guowang Xu, Ping Luo, Yanni Zhao, Yang Ouyang, Xinjie Zhao, Peiyuan Yin, Xin Lu,**Presenting Author Affiliation:** Dalian Institute of Chemical Physics, CAS**Abstract Submission:**

Metabolomics is playing more and more important role in different fields of life science, the nontargeted metabolic profiling analysis is its main characteristic. Because of disadvantage in instrument repeatability and/or analytical throughput, until now most of published studies were limited to less than 180 samples, large-scale metabolomics study, for example, in large prospective epidemiological studies with thousands of samples or in the nontargeted metabolome-wide association study (MWAS) is still difficult although the samples can be separated into small batches, which brings new trouble in data integration.

In this presentation we shall report a comprehensive strategy for large-scale metabolomics studies based on gas chromatography-mass spectrometry (GC-MS) and liquid chromatography (LC)-MS. The core techniques include: 1) pseudotargeted method which mixes the advantages of nontargeted and targeted methods together 2) real and virtual quality control (QC) samples for calibrating gross and systematic errors 3) high throughput not only in metabolite analysis, but also in sample pretreatment 4) post-calibration of metabolic profiling data from different batches and 5) a 'live' database containing LC retention times, MS and MS/MS information of 1500 commonly met metabolites to identify metabolites in the metabolic profiling. Based on this strategy, the repeatability, throughput and profiling information availability are greatly improved. Suggested protocol can be used for large-scale metabolomics studies with thousands of samples based on GC-MS or LC-MS. The liver cancer and plant metabolomics will be used as examples to show the usefulness of the developed strategy.

**Key references**

1. Y. Zhao, et al. (2016) *Anal. Chem.* 88, 2234-2242
2. P. Luo, et al. (2016) *J. Chromatogr. A*, 1437 : 127-136
3. P. Luo, et al. (2015) *Anal. Chem.*, 87 (10): 5050-5055
4. Y. Li, et al. (2012) *J. Chromatogr. A*, 1255: 228-236.

**Poster #: 284**

**Abstract #: 2236**

**Abstract Title:** Application of GC - QExactive to explore fungal/bacterial interactions

**Authors:** Karl Burgess, Stefan Weidt, Jennifer Haggarty, Cristian Cojocariu, Paul Silcock, Gordon Ramage,

**Presenting Author Affiliation:** University of Glasgow

**Abstract Submission:**

Combined infections from *Candida albicans* and *Staphylococcus aureus* are a leading cause of death in the developed world. Evidence suggests that *Candida* enhances the virulence of *Staphylococcus*: hyphae penetrate through tissue barriers, while *S. aureus* tightly associates with the hyphae to obtain entry to the host organism. Indeed, in a biofilm state, *C. albicans* enhances the antimicrobial resistance characteristics of *S. aureus*, with vancomycin concentrations of up to 1600mg/mL being unable to clear the bacteria. The association of *Candida* and *Staphylococcus* is also associated with significantly increased morbidity and mortality. Due to this tight association we hypothesised that metabolic effects were also in evidence.

To explore the interaction, we used a novel GC-Orbitrap – based mass spectrometer, the QExactive GC, which combines the high peak capacity and chromatographic resolution of gas chromatography with the sub-ppm mass accuracy of an Orbitrap system. This allows the capability to leverage the widely available electron ionisation libraries for untargeted applications, along with expanding accurate mass libraries and targeted matches based around authentic standards.

*C. albicans* and *S. aureus* mono- and co-cultured biofilms were analysed in addition to the fresh and spent bacterial growth media. The targeted analysis experiment was based around 36 sugars and sugar phosphates, 22 amino acids and five organic acids. We detected an additional 22 highly scoring compounds from untargeted analysis.

Many of the results were as expected – rapid consumption of glucose and fructose from the medium regardless of the cell type. We also detected trehalose from the untargeted data, only in medium that contained *C. albicans*, commensurate with it being a predominantly fungal sugar. Notable from the results is that the pentose phosphate pathway appears to be enhanced in the cells from co-cultured biofilms.

**Poster #: 286**

**Abstract #: 2336**

**Abstract Title:** Introduction of a quantitative microsampling device

**Authors:** Shinobu Kudoh, Ippei Takeuchi,

**Presenting Author Affiliation:** Shimadzu Techno-Research, Inc

**Abstract Submission:**

A set of microsampling devices was developed to contribute to quantitative metabolomics, bioanalysis and clinical diagnoses. One of the device set is designed to be held easily with fingers to sample 20 or 40  $\mu\text{L}$  of essentially blood utilizing a capillary phenomenon into an inner narrow tubing structure that is coated with an anticoagulant reagent for plasma separation. The other device set is a carousel like holder into which the sampling devices are placed as many as 16 with no concerns of cross-contamination and centrifuged directly. 2.5 or 5  $\mu\text{L}$  aliquots of the resultant serum or plasma are obtained in its two compartment like parts each. The parts are easily plucked off with fingertips and can directly be subjected to sample treatments such as deproteination with no volumetric re-measurement nor further transferring into a volumetric meter for a quantitative measurement. They are durable enough to store in a deep freezer until the day of analysis. The device set is considered to be useful for the sampling of other body fluids such as tears and saliva. Therefore they can be handy devices applicable to many of metabolomics investigations, epidemiological surveys and diagnoses as well as to facilitating 4Rs, Replacement, Reduction, Refinement & our Responsibility for animal studies and to blood sampling from seriously sick patients and neonates or infants where the sampling itself is difficult and lightening their mental and physical burden can greatly be lightened. The sampling accuracy and precision by the device were comparable to those with typical micropipettes.

**Poster #: 287**

**Abstract #: 2373**

**Abstract Title:** Increased Metabolome Identification Coverage Using Optimized LC-MS-MS Conditions on a Tribrid Orbitrap Mass Spectrometer

**Authors:** Reiko Kiyonami, Claire Dauly, Ralf Tautenhahn, David Peake, Ken Miller,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

Metabolomics has become essential for understanding cell biology, physiology and medicine by providing a direct functional readout of cellular biochemical state. Recent advances in UHPLC separation, high resolution accurate mass spectrometers and metabolite database annotations have allowed the rapid and sensitive detection of a variety of metabolites from biological samples with minimal sample preparation. However, metabolite identification from LC-MS based data sets remains challenging due to the presence of many isomeric and isobaric metabolites. In order to confidently identify metabolites, high quality MS/MS or MS<sup>n</sup> data are required for many of the m/z features captured during a metabolomics profiling experiment.

The new Thermo Scientific™ Orbitrap Fusion™ Lumos™ Tribrid™ mass spectrometer offers high sensitivity and fast scan speed (up to 20 Hz), enabling comprehensive coverage of MS/MS spectra of detected m/z features in a LC-MS-MS run. It also enables multiple dissociation techniques, including HCD and CID, to be performed in parallel during a single LC-MS-MS run, providing comprehensive fragment information per m/z feature for confident metabolite identification. Here we present that more than 200 metabolites can be simultaneously identified and quantified from human urine samples using optimized LC-MS-MS conditions on the Orbitrap Fusion Lumos MS. The dedicated untargeted metabolomics software (Thermo Scientific™ Compound Discoverer™ 2.0) is used for confident metabolite identification as well as differential and statistical analysis of the urine sample profiles.



**Poster #: 288**

**Abstract #: 2066**

**Abstract Title:** Multiple isotopomer analysis with Non-uniform sampled NMR for cellular metabolomic studies.

**Authors:** SU JIN LEE,

**Presenting Author Affiliation:** Seoul National University

**Abstract Submission:**

Isotopomer analysis using either  $^{13}\text{C}$ -NMR or LC/GC-MS has been an invaluable tool for studying actual metabolic activities in a variety of systems. However,  $^{13}\text{C}$ -NMR, despite its high resolution, is very insensitive, and MS-based techniques cannot differentiate isotopomers of same molecular mass. In addition, current  $^{13}\text{C}$ -NMR or LC/GC-MS have limitations in detecting metabolites in living cells. Here, we describe a non-uniform sampling-based 2D heteronuclear single quantum coherence (NUS-HSQC) approach to measure metabolic isotopomers in both cell lysates and living cells. The method provided ultra-high resolution that can resolve multiplet structures in the  $^{13}\text{C}$  dimension while retaining the sensitivity of the indirect detection. The approach was actually tested in L1210 mouse leukemia cells labeled with  $^{13}\text{C}$  acetate by measuring NUS-HSQC with 25% sampling density and less than 10 hour experimental time. The results showed a variety of metabolic features such as 1) higher usage of acetate in acetylation pathway than aspartate synthesis through TCA cycle, 2) differential analysis of metabolic intermediates in TCA cycle turns through the analysis of completely resolved malate multiplet, and 3) isotopomers distribution in fatty acids in living cells. Particularly, we were able to detect fatty acid and other lipids along with other hydrophilic metabolic intermediates in a single sample with NMR. Overall the suggested NUS-HSQC approach should provide useful information for carbon flux information in metabolic pathway analysis.

**Poster #: 289**

**Abstract #: 2488**

**Abstract Title:** Internal Extractive Electrospray Ionization Mass Spectrometry for Metabolomics Studies

**Authors:** Huanwen Chen, Hua Zhang, Liang Zhu, Haiyan Lu,

**Presenting Author Affiliation:** East China University of Technology

**Abstract Submission:**

Many currently available analytical mass spectrometry techniques require multi-step sample pre-treatment to obtain molecular information from the 3-dimensional volume of a bulk sample. Commonly associated with sample pre-treatment are biological degradation, chemical reactions, reagent contamination, and material losses. Thus, internal extractive electrospray ionization (iEESI) is established for the straight-forward mass spectrometric analysis of whole-volume (=20 mm<sup>3</sup>) samples (e.g., food, biological tissues) without either mashing/grinding the sample or matrixes clean-up.

Chemical composition (e.g., polyvinyl acetate, glycerol, menthol, menthone, glucose and sucrose) of a chewing gum was characterized by iEESI-MS in a broad mass range ( $m/z$  100–1000) with good signal abundance. The mass spectra recorded by iEESI-MS were remarkably different from those recorded by other ionization techniques including DESI, DAPCI, “leaf spray” ionization, paper spray ionization, etc. Similar to the chewing gum analysis, considerably richer mass spectral patterns for iEESI-MS were also obtained using biological tissue samples including pork meat, garlic leaf, and garlic bulb samples. Our data have shown that iEESI detects analytes located inside a bulk sample, featuring iEESI-MS unique merits to sensitively probe molecular information beneath sample surfaces.

Full scan iEESI mass spectra were recorded from a set (200 samples in total) of 100 lung cancer tissue samples and 100 pericarcinomatous tissue samples. The difference between these samples on the molecular level was detected by iEESI-MS analysis within 2 min which included the time required for sample loading. In the principal component analysis score plot, the each lung cancer sample was clearly distinguished from the corresponding normal ones.

Besides, quantitative analysis of targeted metabolite (e.g., salbutamol in pork meat (3×2×2 cm)) was experimentally demonstrated, showing a linear correlation between the characteristic signal and the concentration of salbutamol in pork meat ( $y=0.6971x+5.79$ ), a good limit of detection (LOD=0.0399 µg/L, S/N= 5) and reasonable relative standard deviation (RSD= 6–15%, n=7).

**Poster #: 290**

**Abstract #: 2519**

**Abstract Title:** Galaxy workflows for mass spectrometry and NMR spectroscopy-based metabolomics

**Authors:** Arturas Grauslys, Ralf Weber, Warwick Dunn, Andrew Jones, Mark Viant,

**Presenting Author Affiliation:** University of Liverpool

**Abstract Submission:**

Background: For metabolomics to achieve its full potential the accessibility, reporting, reproducibility and harmonisation of computational metabolomics tools must be improved significantly. Computational workflows provide one route to achieving such harmonisation. Galaxy is a widely used workflow platform that has helped to transform genomics research by massively increasing the accessibility to powerful data analysis tools. It is intuitive to use and highly flexible allowing non-programmers to create analysis workflows from a broad and expanding suite of tools. Two related Galaxy workflows for metabolomics have recently been reported, the first by a French team (MetaboHub, producing Workflow4metabolomics [Giacomoni et al. *Bioinformatics*. 2015, 31(9):1493-5]) and the second by the University of Birmingham, UK (Galaxy-M [Davidson et al. *GigaScience*. 2016, 5:1-9]). Together these efforts have 'wrapped' several tools for the processing and analysis of NMR, LC-MS, GC-MS and DIMS metabolomics data, increasing their accessibility for the international metabolomics community. However several more tools still require developing and then 'wrapping'.

Results: In a new collaborative project funded by the UK BBSRC to the Universities of Liverpool and Birmingham, we are extending the workflows for analysis of data from mass spectrometry and NMR spectroscopy based-metabolomics experiments. These include developing novel tools as well as integrating existing tools for data processing and analysis, including batch correction, metabolite identification and quantification. We will leverage data formats such as nmrML and develop text-based standards to ensure standardised data flow between tools as well as easy sharing of processed data through public repositories (e.g. MetaboLights). Ultimately we aim to provide a set of well-tested tools that could be easily composed into data analysis workflows that will serve as standard, automated and integrated workflows for mass spectrometry and NMR metabolomics data analysis.

**Poster #: 291**

**Abstract #: 2335**

**Abstract Title:** Ion Mobility/Mass Spectrometry for Metabolomics and Clinical Analysis: Progress and Prospects

**Authors:** Richard Yost, Christopher Chouinard, Michael Costanzo, Michael Wei, Robin Kemperman, Nicholas Oranzi, Timothy Garrett,

**Presenting Author Affiliation:** University of Florida

**Abstract Submission:**

Ion mobility/mass spectrometry has tremendous potential for metabolomics and clinical analysis. Ion mobility can resolve compounds unresolved by LC/MS/MS, provide additional structural information not available from mass spectrometry, and reduce or eliminate the need for chromatographic separation. These features offer significant improvements for quantitative targeted metabolomics and clinical analysis, as well as for untargeted (global) metabolomics studies.

This presentation will explore innovations in ion mobility/mass spectrometry for metabolomics and clinical analysis. Techniques to be covered include both classic drift tube ion mobility (IMS) and high-field asymmetric-waveform ion mobility (FAIMS), in conjunction with MS, MS/MS, and LC/MS. Characterization and optimization of instrumental parameters critical for analytical performance will be explored, including ionization techniques, cationization and complexation of analytes for improved mobility separation, and integration with chromatographic separation and MS/MS. Applications will include a range of metabolomics, lipidomics, and targeted clinical analyses. Specific examples will include rapid clinical assays (vitamin D and its epimers), separation of isomeric performance-enhancing steroids, and breath analysis for early disease screening. Recent advances in these areas will be highlighted, along with a perspective on the metabolomics and clinical future of these approaches.

**Poster #: 292**

**Abstract #: 2273**

**Abstract Title:** A data mining algorithm based on Random Sample Consensus (RANSAC) for the selection of metabolites relevant to strain improvement

**Authors:** Shao Thing Teoh, Sastia Putri, Yukio Mukai, Eiichiro Fukusaki,

**Presenting Author Affiliation:** Osaka University

**Abstract Submission:**

We have previously proposed a metabolomics-based approach for microbial phenotype improvement, where gene modification targets are inferred from data-driven comparison of strains, and applied it to 1-butanol tolerance of *Saccharomyces cerevisiae*. In brief, a set of mutant strains were cultivated, their growth rates under 1-butanol stress were taken as measures of tolerance, and mid-exponential phase cell samples were subjected to snapshot metabolomics by untargeted GC/MS analysis. The metabolite profiles were correlated to 1-butanol stress tolerance performances of each strain by OPLS regression modeling, and metabolites were putatively identified as involved in 1-butanol tolerance based on their PLS Coefficients and VIP Scores. The relevance of these metabolites were validated by successfully predicting new mutant strains with high tolerance based on corresponding metabolic pathways. The results demonstrated the potential of metabolomics in combination with regression modeling in data-driven, semi-rational strain engineering. However, standard PLS could only be used to identify metabolites correlated with the objective phenotype across all samples, whereas it may be possible that some important metabolites may only be well-correlated with the phenotype in a subset of samples. To comprehensively mine metabolomics data for such metabolites, we proposed a data-mining strategy that utilizes Random Sample Consensus (RANSAC) to select subsets of samples with consistent trends for construction of PLS submodels with higher performance (RANSAC-PLS). By applying RANSAC-PLS to the previous dataset, new putative 1-butanol tolerance-related metabolites were identified. Furthermore, the relevance of these metabolites to 1-butanol tolerance could be validated by proposing new high-tolerance strains. The results showed that RANSAC-PLS is a promising strategy to identify unique metabolites providing additional hints for phenotype improvement, which could not be detected by traditional PLS modeling with the entire dataset.

**Poster #: 293**

**Abstract #: 2279**

**Abstract Title:** Novel high-throughput and widely-targeted LC-TOFMS method for D-amino acids  
Metabolomics

**Authors:** Yutaka KONYA, Moyu TANIGUCHI, Eiichiro FUKUSAKI,

**Presenting Author Affiliation:** Osaka University

**Abstract Submission:**

Recently the demand for D-amino acid profiling has been drastically increasing because the significance of D-amino acid in various biological events is suggested. However, the present methodologies for D-amino acid profiling are still unsatisfactory. Therefore, a highly sensitive, robust, high-throughput, and user-friendly method for D-amino acid profiling must be developed. We developed a novel method for D-amino acid profiling using a combination of a chiral column and time of flight mass spectrometry (TOFMS).

To our knowledge, our approach has the best performance for D-amino acid simultaneous analysis that includes,

1. The shortest analytical time (within 10 minutes for proteionogenic amino acids)
2. The highest enantioseparability without derivatization
3. The largest coverage for analytical targets (totally more than one hundred targets including nonproteionogenic amino acids and amines).

In the past, there were few metabolomics researches which focused on D-amino acids. Thus, our novel profiling method will be instrumental in advancing the D-amino acid research in the future.

**Poster #: 294**

**Abstract #: 2294**

**Abstract Title:** Single sample analysis for proteomics and metabolomics

**Authors:** Joanna Godzien,

**Presenting Author Affiliation:** CEMBIO, CEU San Pablo University

**Abstract Submission:**

Having low volume samples can reduce the possibility of combining different omics techniques to answer biological questions. However, it is clear that multi-omics approaches offer a huge advantage in gathering wider, relevant information from any sample. To overcome this serious lack, an extensive investigation has been performed to develop a new method for lipidomics/metabolomics and proteomics analyses in sequence from a single, sample with volume as low as 60  $\mu$ L. For this purpose in-vial dual extraction (IVDE) was devised to analyse HDL and LDL fractions from single plasma samples.

IVDE leads to the formation of three phases: ether lipophilic upper-phase, aqueous hydrophilic mid-phase and protein pellet lower-phase. This innovative method allows the separation of these phases within an insert of HPLC vial from which samples can be injected directly from by adjustment of the position of the instrument's injection needle. Firstly, lipidomics analysis was performed in both ionisation modes, after which the protein pellet was used for proteomics analysis, together revealing a huge array of information about the sample. Comparing direct sample preparation for proteomics to this method following metabolomics/lipidomics analyses revealed no differences. Furthermore, the protein pellet was lipid free which is highly important for the proteomics measurements.

The robustness and reliability of the methodology proposed was tested firstly analysing HDL and LDL fractions, comparing and contrasting the information obtained with knowledge of their composition. Secondly this method was applied to an animal model of WT and ApoE knockout mice to test its ability in detecting biological changes occurring in an organism. It was clearly proven that IVDE can be used to detect alterations occurring in both lipids and proteins. This method provides huge scope in multi-omics analyses, which can be differently combined to study diverse aspects of single low volume samples, not limited to lipidomics and proteomics.

**Poster #: 295**

**Abstract #: 2440**

**Abstract Title:** Globally Optimized Targeted Mass Spectrometry (GOT-MS): Reliable Metabolomics Analysis with Broad Coverage

**Authors:** Daniel Raftery, Haiwei Gu,

**Presenting Author Affiliation:** University of Washington

**Abstract Submission:**

Global profiling and targeted mass spectrometry (MS) methods play important roles in metabolomics for detecting and quantifying metabolites. However, these methods have various limitations, including missing metabolite signals or limited metabolome coverage. We present a new method, globally optimized targeted (GOT)-MS, that broadens the coverage of targeted MS, including unknown metabolites, while maintaining excellent reproducibility.

The key step in GOT-MS is a global search of precursor and product ions in order to optimize the detection capabilities of LC-QQQ. Aqueous metabolites were extracted from a serum sample and separated on a HILIC column. We first performed selected ion monitoring (SIM) incremental scanning, and the  $m/z$  values that produced good peak shapes and S/Ns were selected as precursor ions. We then carried out tandem mass spectrometry (MS/MS) scanning with incremental collision energy (CE) to profile product ions. With both precursor and product ions, MRM scanning was used to optimize the instrument parameters. From the aqueous serum extracts serum, 595 GOT-MS precursor ions and 1,890 MRMs were determined. The average intraday CV for all 1,890 GOT-MS MRMs was  $7.8 \pm 7.0\%$ , and the average interday CV for amino acids was  $8.3 \pm 3.4\%$ . For the many detected MRMs, the analytical performance of GOT-MS is comparable to or better than a Q-TOF instrument of similar vintage. There are also many fewer missing metabolite signals and higher dynamic range using GOT-MS.

We evaluated the performance of GOT-MS for biomarker discovery using serum samples from 20 colorectal patients and 20 matched healthy controls. We found 26 GOT-MS metabolite signals with fold changes (FCs)  $>2$  and  $P < 0.05$ . Using the Metlin database, over 900 compounds were identified, indicating that GOT-MS is well qualified to detect not only well-known metabolites but also unknowns.



**Poster #: 296**

**Abstract #: 2446**

**Abstract Title:** Segmenting of mass range improves metabolite identification for Exactive type mass spectrometers

**Authors:** Fedor Kryuchkov, Mathias Ziegler, Frank Kjeldsen,

**Presenting Author Affiliation:** UiB

**Abstract Submission:**

Recently, Exactive family instruments have become very popular in metabolomics due to their high resolution and high mass accuracy capabilities. For such analysis instrument needs to be run in MS1 mode. General recommendations are to run instrument in full mass spectrum mode and at highest resolution in positive or alternatively in positive and negative mode. This method does not provide highest sensitivity due to limited dynamic range caused by space effects of Orbitrap mass analyzer. To achieve highest sensitivity instrument needs to be utilized in selected ion monitoring mode, but in this case information about other ions will be lost. To find a balance between sensitivity and diversity, mass range can be segmented. Limiting factor of this approach is speed of mass spectra acquisition, because instrument needs to scan enough points for acceptable quantification. Chromatographic separation of water soluble metabolites is usually done by hydrophilic interaction liquid chromatography (HILIC) and this method usually provides wider peaks than methods based on reverse phase separation. In the current work, we used ZIC-pHILIC polymeric (5 $\mu$ m) 150  $\times$  2.1 mm column. In the Full MS mode, QExactive can record up to 50 MS spectra for one LC peak at highest (140,000) resolution. This speed is three to five times higher than needed for reliable quantification. Here we suggest applying segmentation of mass range while sacrificing excessive speed. To construct proper pattern, the first step is to run quality control sample (i.e. mixture of all samples to be analyzed) in Full MS mode at highest resolution. After that, the acquired peak list is analyzed by custom algorithm which constructs mass range segmentation pattern. Further, this pattern is loaded as MS method, and it is further used to analyze samples. Performance of this data acquisition modification is evaluated by Thermo Sieve program and yeast metabolite extract.

**Poster #: 298**

**Abstract #: 2564**

**Abstract Title:** Doubling metabolite coverage in untargeted metabolomics of human plasma

**Authors:** Dajana Vuckovic, Dmitri Sitnikov, Hanieh Peyman, Parsram Ramrup,

**Presenting Author Affiliation:** Concordia University

**Abstract Submission:**

Methanol and methanol/ethanol solvent precipitations are currently the most common sample preparation methods for global metabolomics of human plasma using LC-MS because of their wide metabolome coverage and excellent repeatability. However, the main two disadvantages of these methods are that low abundance metabolites are routinely not detected and high potential for matrix effects. In this research, I will summarize our efforts to double metabolome coverage for human plasma without increasing LC-MS analysis time and compare/contrast different approaches that can be used to achieve this goal in shortest analysis time. Sequential extraction, liquid-liquid extraction using ionic liquids and dispersive solid-phase microextraction using hydrogel materials will be compared for their performance for untargeted metabolomics of plasma. The effect of these different sample preparation strategies on metabolite coverage and method precision will be discussed. We tested different types of extraction phases including commercial core-shell nanoparticles (CERES Nanotrap) with acrylic acid or cibachron blue cores and hydrogel microparticles functionalized with vinyl acetate, acrylic acid or N-3-aminopropyl methacrylamide hydrochloride. For ionic liquids, we tested both pyrrolidinium and imidazolium ionic liquids with varying carbon chain lengths and varying anion combinations in order to help determine the contribution of anion and cation to the extraction mechanism across different metabolite classes. We will show that functionalized hydrogels and ionic liquids represent novel extraction materials that can improve both targeted and untargeted sample extraction for metabolomics studies of human plasma. This is the first systematic investigation of these materials across different metabolite classes.

**Poster #: 299**

**Abstract #: 2355**

**Abstract Title:** Biochemical changes associated with retinoic acid-induced differentiation of SH-SY5Y human neuroblastoma cells

**Authors:** Garth Maker, Alicia Manning, Ian Mullaney, Robert Trengove,

**Presenting Author Affiliation:** Murdoch University

**Abstract Submission:**

SH-SY5Y neuroblastoma cells have been widely used to model neurotoxic processes. It is unclear how well, in their undifferentiated, immortalised state, the cells model the original tissue. SH-SY5Y cells can be differentiated with retinoic acid (RA), to become more 'tissue-like', and potentially better reflect the biochemistry of a mature neuronal cell. This study used untargeted GC-MS metabolomic analysis to investigate the downstream biochemical changes in a RA-differentiated cell, and how these processes were affected by exposure to malaoxon, a toxic metabolite of the pesticide malathion, as a model for toxicology research.

Cultured human SH-SY5Y neuroblastoma cells were exposed to 10  $\mu$ M RA for 120 hours and 1  $\mu$ M malaoxon for 24 hours. The samples were quenched, harvested, extracted and derivatised before metabolomic analysis was carried out using a Shimadzu QP2010 GC-MS. The metabolites that contributed most to the variance were identified using PCA. This indicated that RA-differentiated cells, and undifferentiated and differentiated cells exposed to malaoxon, were grouped in relation to metabolite profile. Changes were profiled in a range of metabolites including amino acids, carbohydrates, fatty acids and unknowns, indicating that exposure to RA caused a biochemical response in relation to neurite outgrowth, survival and apoptosis, decreased energy demand for cell proliferation and increased synthesis and activation of proteins involved in cell signalling. Data suggested that exposure to malaoxon induced a glutamate-mediated excitotoxic response causing oxidative stress, mitochondrial dysfunction, energy depletion and damage to the cell membrane.

The data indicates that there were a range of biochemical changes associated with a RA differentiated phenotype. Induction of survival and apoptotic responses may have facilitated a neuroprotective response that decreased the toxic effects of malaoxon. The outcomes of this study have implications for research using differentiated SH-SY5Y cells, with the decision to use them resting on the neurotoxic model being investigated.

**Poster #: 300**

**Abstract #: 2331**

**Abstract Title:** National Institutes of Health Common Fund- Metabolomics Community Resources

**Authors:** Padma Maruvada, David Balshaw, Arthur Castle, Danthi Simhan, Leslie Derr, Chanda Dutta, Yih-Woei Fridell, Katrnia Gwinn, Conrad Mallia, Aaron Marquitz, Gary Murray, Andrew Maynard, Laurie Nadler, Richard Okita, Steven Oversby,

**Presenting Author Affiliation:** National Institute of Diabetes, and Digestive and

**Abstract Submission:**

The NIH Common Fund recently invested in addressing technical limitations and building the national capacity for metabolomics research. The Metabolomics Program includes 6 Regional Comprehensive Metabolomics Resource Cores (RCMRCs), 6 technology development grants, a Data Repository and Coordination Center (DRCC) at UCSD, ten mentored research awards, and two education project grants to design metabolomics courses. In addition, many collaborative projects have been funded that apply metabolomics technologies to biomedical research. As it enters the fourth year of grant funding, the Program supports a wide range of resources and capabilities. This presentation will provide an overview of the research resources supported by the NIH Common Fund Metabolomics Program including core facilities, data repository coordination, technology development, metabolite standards synthesis, and workforce development. In addition, there will be discussion on how these resources can be leveraged in order to advance international metabolomics research.

**Poster #: 301****Abstract #: 2374**

**Abstract Title:** Identification of biomarkers associated with early psychotic disorder (ages 12 and 18) in the Avon Longitudinal Study of Parents and Children (ALSPAC) cohort using a lipidomic approach.

**Authors:** Aoife O'Gorman, Mary Cannon, Lorraine Brennan, UCD David Cotter, Matej Oresic, Tuulia Hyotylainen, Tommi Suvisaari, Stanley Zammit,

**Presenting Author Affiliation:** Royal College of Surgeons in Ireland

**Abstract Submission:**

Recent clinical studies suggest that early intervention alleviates progression and improves therapeutic outcomes in psychotic illnesses. Biological predictors of early psychosis will be of enormous clinical value. Global profiling strategies such as unbiased lipidomics may hold a significant potential for translating discriminatory molecules into clinical biomarkers. Therefore, the objective of this study was to apply a lipidomic approach to identify biomarkers of psychotic disorder (PD) at ages 12 and 18 in the ALSPAC cohort.

Fasting plasma lipid profiles of healthy controls were compared with samples of subjects with PD both at ages 12 and 18 in the ALSPAC cohort. PD was defined as those participants who at age 12 or 18 had experienced a definite clinical psychotic experience, which were identified through face-to-face, semi structured Psychosis-Like Symptom Interview (PLIKSi) conducted by trained psychology graduates in assessment clinics.

Censored regression analysis was used to identify significantly discriminatory metabolites between the control and PD groups. Multivariate statistical approaches such as principal component analysis (PCA) and hierarchical cluster analysis (HCA) were applied to identify and evaluate potential biomarkers of PD.

A total of 179 lipids were identified in the plasma samples. Censored regression identified 31 significant lipids ( $P < 0.05$ ) between controls and the PD group at age 12, of which seven remained significant at *fd*r level. HCA analysis identified 7 clusters, 3 of which were significantly increased in the PD group. These significant clusters contained (1) LPCs, (2) PCs and (3) PCs and CEs. At age 18, censored regression identified 23 significant lipids ( $P < 0.05$ ) between controls and the PD group, none were significant at *fd*r level. HCA analysis identified 7 clusters, one was significantly decreased in the PD group, this cluster contained LPCs.

Further work is required to validate these clusters of lipids and examine their potential as biomarkers.

**Poster #: 302**

**Abstract #: 2558**

**Abstract Title:** Comparison of different analytical platforms for non-targeted metabolomics of cancer cells

**Authors:** Michaela Schwaiger, Karin Ortmayr, Gerrit Hermann, Kristaps Klavins, Evelyn Rampler, Walter Miklos, Walter Berger, Gunda Koellensperger,

**Presenting Author Affiliation:** University of Vienna, Analytical Chemistry

**Abstract Submission:**

The emergence of LC-MS based non-targeted metabolomics leads to large datasets containing information for a multitude of intracellular metabolites. Screening tools are typically employed within a differential approach aiming at finding global changes in biological systems for hypothesis generation and corroboration. However, these findings intrinsically depend on the coverage and selectivity of the applied analytical methods. Efficient and robust chromatographic separations are indispensable, especially where high-resolution mass spectrometers are challenged by isomeric and isobaric primary metabolites.

Applying reversed phase and hydrophilic interaction chromatography only, will result in metabolic fingerprints only partially reflecting such important pathways as e.g. energy metabolism, purine and pyrimidine salvage pathways. Essential primary metabolite classes such as sugar phosphates are not separated despite the orthogonality of the LC separations. In order to tackle this analytical challenge, the power of graphitized carbon and ion chromatography coupled to high resolution mass spectrometry in non-targeted workflows is assessed. The methods are implemented as one-dimensional separations and as on-line combination to orthogonal reversed phase chromatography. The presented on-line combinations provide a broad coverage across different compound classes within one analytical run. A comparison of the different measurement platforms will be performed using a cancer cell model of acquired resistance investigating sensitive versus resistant cells. Biological repeatability will be addressed by comparing completely independent experiments. Moreover, the findings will be validated by absolute quantification of 188 metabolites including amino acids and acylcarnitines.

**Poster #: 303**

**Abstract #: 2485**

**Abstract Title:** Sequential Detection of Metabolites, Lipids and Proteins in a Bulk Tissue Using Internal Extractive Electrospray Ionization Mass Spectrometry

**Authors:** Haiyan Lu, Hua Zhang, Wei Zhou, Yiping Wei, Huanwen Chen,

**Presenting Author Affiliation:** East China University of Technology

**Abstract Submission:**

Obtaining metabolites, lipids and proteins from a single biological tissue sample significantly increase the amounts of chemical information, which provides multi-dimensional insights for better understanding the biochemical metabolism and pathophysiological process[1]. Usually, mass spectrometric studies on metabonomics, lipidomics, and proteomics may be performed separately using individual tissue samples. This strategy works perfectly with the cost of large amount of time and tissue samples. For integrated systemic biology studies, it is highly desirable to fast probe the molecular information with minimal sample analysis time and operation interferences. Herein a novel method to sequentially detect metabolites, lipids, and proteins in a single tissue sample has been proposed using internal extractive electrospray ionization mass spectrometry (iEESI-MS)[2-4].

In this study, using the same iEESI setup and the same piece of pig's lung tissue, the mass spectral patterns were changed dramatically when different solvents were infused. In combination of the full scan MS and tandem MS data, it was evident that large amounts of the small metabolites, lipids and proteins were sequentially detected in the low mass range ( $m/z$  100-250), middle mass range ( $m/z$  700-900) and relatively high mass range ( $m/z$  900-1300) when the extraction solution was changed from either methanol/water (v/v, 35/65) or methanol/water/acetic acid (v/v/v, 35/65/2.5) to methanol/water/acetic acid/acetone (v/v/v/v, 35/65/2.5/5). These spectral features were reproducibly obtained, showing that the mass spectral signals were high correlated with solvents used for internal extraction and electrospray ionization.

Besides, this method have been successfully applied to rapid differential analysis of tiny amounts of human lung tissue samples with significantly improved accuracy, providing a novel method to fast identification the edges of a mutated tissues at the molecular level. Although it is only a starting point, the method shows great potential applications in clinical surgical operations.

**Poster #: 304**

**Abstract #: 2554**

**Abstract Title:** SelexION-based removal of detergent interference for shotgun-lipidomics

**Authors:** Cyrus Papan, Joerg Dojahn, Hartmut Michel, Julian Langer,

**Presenting Author Affiliation:** AB Sciex Germany

**Abstract Submission:**

Mass spectrometry employing electrospray ionization is intrinsically plagued by chemical interference problems, adversely affecting analyte detection. Major sources of interference are surfactants and detergents, which are often required for e.g. stabilization of membrane proteins or efficient tissue lysis. This issue is particularly critical in direct-infusion based applications, e.g. in shotgun lipidomics. SelexION® Differential Mobility Spectrometry (DMS) technology filters ions based on their differential mobility in an oscillating electrical field with an asymmetric wave form. Here we show that SelexION® technology is capable of filtering detergents from the ion beam before they enter the MS orifice, allowing direct analyses of lipids in detergent-containing samples.

We conducted a systematic survey for separating multiple detergents and different lipid classes. We tested the detergent:lipid separation in a low-complexity sample (using detergents with specific lipid classes) and a near-physiological, complex lipid sample. To this end, a commercially available bovine heart total lipid extract was supplemented with typical working concentrations of detergent. Using the SelexION technology device, we directly infused the mixtures and ramped the compensation voltage (CoV) over a range of - 40V to +30 V, the range in which lipids normally separate. The extracted ion chromatogram shows that most peaks represent, as expected, the different lipid classes while one distinct peak almost exclusively contains the detergent species, thus allowing separate detection of lipid and detergent molecules. We now perform systematic analyses of different detergent classes including glycosides, maltosides and anionic detergents, cholate, digitonin, triton-derivatives and new detergent classes. This methodology could be a valuable new tool for directly analysing the lipid content of samples that require a high concentration of detergent.



**Poster #: 305**

**Abstract #: 2332**

**Abstract Title:** Determining polar metabolites using high throughput ion chromatography (IC) coupled with high resolution accurate mass spectrometry (HRAM)

**Authors:** Terri christison, Junhua Wang, Ken Cook, Ryo Komatsuzaki, Linda Lopez, David Peake,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

TCA and glycolysis metabolites are important to understanding the metabolism-related disease mechanisms, however the analysis of these small polar metabolites can be challenging. The metabolites are often isobaric isomers with the same  $m/z$  and fragmentation spectra, requiring chromatographic separation. The common separation methods, such as reverse phase and HILIC, have difficulties separating ionic compounds. It was previously reported that capillary ion chromatography (CapIC) separations had 10 to 100-fold higher sensitivities than other separation methods (HILIC and RP) as a result of the low chemical background from the suppressor technology. The CapIC method also demonstrated superior separations over HILIC and RP by resolving 11 isomeric monophosphate sugars and nine diphosphate sugars in cell lysates. These results were repeated using an analytical IC system running 20-min gradient, demonstrating the potential of high throughput IC separations.

Here we demonstrate a fast 9-min assay for targeted analysis of large sample sets by multiplexing on a dual ICS-5000+ HPIC IC system with an additional 10-port valve. Multiplexing was facilitated by a simple “if then” program and the interfacing software SII for Xcalibur to couple Xcalibur with Chromeleon 7 IC operating system. Using multiplexing, one system pump and eluent generator cartridge generate the KOH gradient at 0.38 mL/min to control the separation conditions for the column in line with the conductivity detector, desalting suppressor, and the Q Exactive MS detector. The second pump and eluent generator provide equilibration conditions at the same flow rate for the offline column. The gradient was optimized to separate glucose 6-phosphate and fructose 6-phosphate and verified by the separation, response, and reproducibility of the six stable isotopic labeled (SIL) standard. The results showed baseline resolution of citrate-isocitrate and trans-cis aconitate isobaric pairs with minimal resolution loss for the isobaric mono- and di-phosphate sugars in the cell lysate samples.

**Poster #: 306**

**Abstract #: 2274**

**Abstract Title:** Carbon metabolism in sugar beet leaves experiencing heat stress

**Authors:** Clémence de Jaham,

**Presenting Author Affiliation:** INRA Bordeaux

**Abstract Submission:**

Sugar beet is the second major plant for sugar production with 22% of the world production in 2012. Mostly cultivated in temperate areas like Northern Europe, sugar beet is sensitive to heat stress. For farmers and sugar refineries this leads to a decrease in sugar yield when the average day temperature is increased by only a few degrees. In the sight of global warming and in order to deliver varieties that exhibit competitive yields in warmer conditions, breeders need a better understanding of sugar beet whole plant response to heat stress. Within this study, five genotypes – a high sugar content genotype (18% of sucrose in the root), a high yielding genotype (17% of sucrose in the root) and its 2 parental lines, and a genotype better adapted to heat stress (comparatively higher sugar yield under warmer climates) - were cultivated in controlled and stressing conditions (22°C /18°C and 30°C/18°C day/night temperatures, respectively). For each genotype, samples of sink and source leaves were harvested from 6-week-old plants every 4 hour in a 24h diurnal cycle. Metabolites and enzymes of central metabolism were measured on microplates and/or via NMR spectroscopy. Metabolite contents showed strong changes depending on the treatment. In source leaves, most metabolites decreased under stress, except sucrose content which was equivalent to control condition. In sink leaves, carbon metabolism compounds seemed to increase under stress, while nitrogen metabolism compounds decreased. The most striking result was that diurnal changes in the carbon balance, particularly sucrose turnover, showed differences in source and sink leaves according to genotype and culture conditions.

**Poster #: 307**

**Abstract #: 2219**

**Abstract Title:** Metabolomic study of tolerance to water limitation for sunflower germination

**Authors:** Thierry BERTON, Stephane BERNILLON, Mickael MAUCOURT, Catherine DEBORDE, Natalia FALAGAN, Marine SAUX, Hayat BOUTEAU, Christophe BAILLY, Annick MOING,

**Presenting Author Affiliation:** INRA Bordeaux

**Abstract Submission:**

Climate change is now recognised as one of the most serious challenges facing the world. In particular, climate change is a challenge for farming, and water limitation is a major abiotic stress which affects plant growth and farming yield. In this way, agricultural adaptation is necessary for the future and we need to understand why some varieties are more resistant or more responsive to environmental conditions.

In this context, tolerance to water limitation during sunflower (*Helianthus annuus*) germination was studied. Two hybrids, one tolerant and one sensitive, were selected and treated under two different conditions:

- 15h control seed imbibition with water,
- 15h seed imbibition with PEG to simulate a water stress.

A non-targeted metabolomic study was then realised, based on liquid chromatography coupled to mass spectrometry (LC-MS) and proton nuclear magnetic resonance spectroscopy (1H-NMR). 1H-NMR spectra and the main compounds of MS spectra were annotated. Thus, 47 major compounds were selected and univariate and multivariate statistical analyses were realised on these compounds. Statistical analyses were also realised on the entire MS profiles.

These analyses have demonstrated a great difference between the two hybrids and the results obtained have shown a difference clearly more important between the two hybrids than between the two treatments. The effect of PEG imbibition was also investigated for each hybrid. We observed more response markers for the tolerant hybrid than for the sensitive one, showing that the tolerant hybrid seems more responsive to PEG imbibition. The metabolomics data will be combined with proteomic and transcriptomic data.

Acknowledgements: SUNRISE (ANR-11-BTBR-0005), MetaboHUB (ANR-11-INBS-010) projects.

**Poster #: 308**

**Abstract #: 2387**

**Abstract Title:** LC-MS-based metabolite footprinting: Application to the characterisation of mesenchymal stem cell differentiation

**Authors:** Amal Surrati, Rober Linforth, Ian Fisk, Virginie Sottile, Dong-Hyun Kim,

**Presenting Author Affiliation:** Wolfson Centre for Stem Cells, Tissue, Engineering

**Abstract Submission:**

Bone regeneration is a complex biological process where major cellular changes take place to support the osteogenic differentiation of mesenchymal bone progenitors. The characterisation of these biological changes and better understanding of the pathways regulating the formation of mature bone cells will increase the therapeutic potential of mesenchymal stem cells (MSC) in the fields of regenerative medicine and drug discovery. Although cell phenotyping methods such as gene expression analysis, protein immunodetection and flow cytometry have been established, these approaches includes invasive and cell distractive steps which prevent monitoring live cells. Since liquid chromatography (LC)-mass spectrometry (MS)-based metabolite footprinting has become a powerful approach to understand the microenvironment of a cell of interest, this technique was used for the investigation of metabolic changes occurring in the culture medium during MSC osteogenic differentiation non-invasively. Mineral deposition and alkaline phosphatase activity, which are two hallmarks of osteogenesis in vitro, were investigated in parallel.

Here we present the first global metabolite footprinting with uni- and multivariate analysis for the non-destructive characterisation of MSC differentiation. This non-invasive approach demonstrated significant metabolomic changes between the media from control and OS-treated cells showing distinct effects of MSC differentiation on the environmental footprint of the cells in different conditions (control vs OS treatment). A subset of compounds was directly linked to the osteogenic time-course of differentiation, and represent interesting metabolite candidates as non-invasive biomarkers for characterising the differentiation of MSCs in a culture medium.

**Poster #: 309**

**Abstract #: 2414**

**Abstract Title:** Metabolomic profiling of *Fraxinus excelsior* genotypes tolerant or susceptible to ash dieback disease reveals changes in specific glycosides.

**Authors:** Christine Sambles, Hannah Florance, Deborah Salmon, Thomas Howard, Nicholas Smirnoff, Lene Rostgaard Nielsen, Erik Dahl Kjær, David Studholme, Murray Grant,

**Presenting Author Affiliation:** University of Exeter

**Abstract Submission:**

European common ash, *Fraxinus excelsior* L., is currently under serious threat of elimination from ash dieback disease (ADB) caused by the fungus, *Hymenoscyphus fraxineus*. To detect and identify metabolites that may be products of pathways important in contributing to resistance against *H. fraxineus*, untargeted metabolomic profiling was undertaken on leaves from strains of *F. excelsior* tolerant or susceptible to *H. fraxineus*. This approach identified sets of features that enabled the discrimination between tolerant or susceptible genotypes of *F. excelsior*. Notably, we observed a decrease in abundance of compounds involved in plant defences against herbivores in ADB tolerant genotypes, suggesting trees tolerant to ADB may have greater susceptibility to herbivorous attack. As plants are often under attack from both herbivores and fungal pathogens, selecting genotypes for future planting that are tolerant to multiple sources of attack are critical for sapling selection. The main herbivorous threat to *F. excelsior* is the emerald ash borer (EAB), which has devastated *Fraxinus pennsylvannica* populations in the United States and Russia whereas the Asian Manchurian ash (*Fraxinus mandshurica*) is generally tolerant to attack. Although EAB has not yet reached the UK, by selecting *F. excelsior* genotypes that are tolerant to *H. fraxineus* for re-establishment of ash forests we may be unwittingly selecting genotypes with a greater susceptibility to EAB. In conclusion, untargeted metabolomic profiling has enabled the discrimination between genotypes of *F. excelsior* that are tolerant or susceptible to the ascomycete, *H. fraxineus*, the causative agent of ash dieback disease and highlighted the possible danger of selective breeding in response to a single pest or disease.

**Poster #: 310****Abstract #: 2012**

**Abstract Title:** Targeted analysis of primary- and secondary-metabolites, and phytohormones from a single plant extract – a method accounting for complexity in plant metabolomics

**Authors:** Martin Schafer, Christoph Brutting, Mario Kallenbach, Gordon van 't Slot, Magdalene Kutyniok, Ian Baldwin,

**Presenting Author Affiliation:** MPI for Chemical Ecology, Jena, Germany

**Abstract Submission:**

Continuous advances in analytical instrumentation allow many laboratories to routinely analyze even subtle parts of the plant metabolism. Many methods were developed e.g., for targeted analysis of plant hormones, primary metabolites and particular toxic plant compounds. However, although it is known that most of these metabolites can be connected to each other they are hardly analytically addressed together and would require various time consuming sample preparations.

Here we present a procedure that allows for the coordinated quantification of more than hundred plant metabolites based on a single solid-phase extraction sample preparation and analysis using a UHPLC-TQ-MS/MS system (Bruker EVOQ Elite). It enables the analysis of high abundance compounds that require only few sample preparations, as well as for low abundance compounds that need intense cleanup and concentration steps.

We used *Nicotiana attenuata*, wild tobacco, to show the applicability of the method. The extensive content of nicotine and other secondary metabolites renders *N. attenuata* extracts a challenging matrix for metabolomic approaches. Exemplary the response of plant leaves to simulated herbivore attack, as well as metabolic differences in tissues related to flower and seed development will be shown. We measured various phytohormones (e.g., abscisic acid, auxins, jasmonates, salicylic acid, cytokinins and gibberellins), primary metabolites, like amino acids and sugars, and specialized metabolites, including particular phenolamides, flavonol-glucosides and alkaloids, as well as their precursors. The data illustrate that both processes involve complex rearrangements in plant metabolism, which are not restricted to the classical defense and growth related sectors, respectively. Importantly, the method showed to be suitable for the analysis of small tissue amounts (10-100 mg fresh mass), up to 192 samples can be prepared in less than 2 working days and can be performed in the 96-well format, which allows its application for high-throughput screenings.

**Poster #: 311**

**Abstract #: 2053**

**Abstract Title:** Fruit position within pear trees impacts ripening and associated metabolism after harvest

**Authors:** David Rudell,

**Presenting Author Affiliation:** USDA-ARS

**Abstract Submission:**

The characteristics of fruit ripening can contribute to the overall quality of the final product. Ripening of European pears (*Pyrus communis*) is impacted by a combination of cultural practices and postharvest storage conditions. Fruit position within a tree canopy can alter fruit development and ripening after harvest. Whether that tree position would, likewise, impact overall fruit metabolism associated with ripening and fruit flavor and quality was the subject of this research. 'd'Anjou' pear fruit harvested from internal and external portions of tree canopies of large, open vase trained trees were stored under a hypoxic controlled atmosphere at -0.5 °C for up to 8 months. We employed multiple GC and LC-MS approaches, accounting for metabolites of a wide range of polarity and volatility, to track dynamic metabolic changes occurring alongside ripening under these conditions. PCA models indicated the estimated metabolomes of external and internal fruit were different at harvest and throughout storage. A PLS model was used to link a number of metabolites including those contributing to aroma and other flavor components with a particular tree position that would impact on-shelf fruit quality. Correlation networks indicated multiple potential areas of co-regulation of these and other metabolites indicating differential coordination of fruit quality-related metabolism. Pathways included phytosterol conjugation, lipid composition, aroma volatile production, sugar metabolism, and acid metabolism. Moreover, results indicate that tree position not only alters the rate at which fruit ripens, but also ripening characteristics, potentially impacting the consistency of the product throughout the commercial supply chain.

**Poster #: 312**

**Abstract #: 2207**

**Abstract Title:** Searching for marker metabolites of crop performance

**Authors:** Annick Moing, Maria Urrutia, Olivier Fernandez, Vanessa Zhendre, Nadia Lamari, Stephane Bernillon, Mickael Maucourt, Catherine Deborde, Daniel Jacob, Patricia Ballias, Helene Sellier, Isabelle Quillere, Bertrand Hirel, Nicolas La

**Presenting Author Affiliation:** INRA Bordeaux

**Abstract Submission:**

For crop plants, metabolic phenotypes, which are positioned between gene expression and highly complex traits such as yield or other components of crop performance and provide condensed information, represent a good opportunity to search for biomarkers. Thus, measurements of single metabolites or small groups of metabolites could be used to predict plant performance under optimal or stressing growth conditions.

This approach has been initiated for two crops, maize and sunflower, and several biological targets within two national projects gathering private and public partners with multidisciplinary expertise (AMAIZING <http://www.amaizing.fr/> and SUNRISE <http://www.sunrise-project.fr/>). Core panels of several tens of well characterized and genetically diverse genotypes are grown in the field or in phenotyping platforms under control or abiotic stress conditions in order to assess the metabolic differences induced by stress. Metabolomics profiling of leaf extracts using liquid chromatography mass spectrometry of semi-polar extracts and proton nuclear magnetic resonance of polar extracts is used to evaluate the metabolic changes. Multivariate and univariate statistical analyses are used to compare the leaf metabolic profiles of plants grown under normal and stress conditions. The metabolite or metabolite signature data are combined with plant phenotyping data in order to search for response biomarkers. Once identified, potential biomarkers will then be analyzed using high-throughput targeted methods on larger genotype panels, opening the way to GWAS. This strategy will be illustrated using several examples including studies of chilling or water limitation responses.

Acknowledgements: SUNRISE (ANR-11-BTBR-0005), AMAIZING (ANR-10-BTBR-01), MetaboHUB (ANR-11-INBS-010) and PHENOME (ANR-11-INBS-012) projects.



**Poster #: 313****Abstract #: 2139**

**Abstract Title:** High throughput metabolomics and hyperspectral imaging of greenhouse grown maize plants reveal response to nitrogen treatments and predict field yield

**Authors:** Jan Hazebroek, James Janni, April Agee Carroll, Roland Welle, Richard Fox, Teresa Harp, Chris Vlahakis, Jian Jin,

**Presenting Author Affiliation:** DuPont Pioneer

**Abstract Submission:**

DuPont Pioneer assesses performance of seed products under development with extensive multiple location field trials. This approach is complicated by environmental differences across years and locations that affect plant growth, response to stresses, and seed yield. Field testing also requires considerable investments in land and time. Clearly, screening product candidates prior to field testing would have value if such a screen is able to predict favorable and/or unfavorable field performance. Towards this end, we applied high throughput metabolomics and hyperspectral imaging to a large scale experiment comprised of greenhouse grown maize plants treated to different levels of inorganic nitrogen. One hundred fifty-nine experimental maize genotypes along with control hybrids were grown from seed in an automated greenhouse according to a partially balanced incomplete block design. Five different nitrogen treatments were applied as the plants grew. Spatial and temporal randomization coupled with periodic movement reduced bias attributable to planting date and physical location in the greenhouse. Plants were imaged at each of two developmental stages and leaves were sampled for metabolomics at one. Metabolomics samples were extracted and analyzed by GC/MS and DI/MS using established sample prep, data acquisition, and data processing protocols. Data were normalized using a mixed model schema that corrected for many causes of variation emanating from both greenhouse and analytical processes that were orthogonal to genotype and treatment. A partial least squares (PLS) model built from GC/MS data obtained from control hybrids was highly linear across all five nitrogen treatments. This model was applied to data from the 159 experimental genotypes to assess its predictive power for field yield of these same entries. PLS was also utilized to model field yield directly from data from greenhouse plants. Our pilot experiment demonstrated the potential for metabolomics and hyperspectral imaging to predict crop field performance.

**Poster #: 314**

**Abstract #: 2481**

**Abstract Title:** Landscape of the lipidome and transcriptome under heat stress in *Arabidopsis thaliana*

**Authors:** Yasuhiro Higashi, Yozo Okazaki, Fumiyoshi Myouga, Kazuo Shinozaki, Kazuki Saito,

**Presenting Author Affiliation:** RIKEN CSRS

**Abstract Submission:**

Environmental stress causes membrane damage in plants. Lipid studies are required to understand the adaptation of plants to climate change. Here, LC-MS-based lipidomic and microarray transcriptome analyses were carried out to elucidate the effect of short-term heat stress on the *Arabidopsis thaliana* leaf membrane. Sixty-six detected glycerolipid species were classified according to patterns of compositional change by Spearman's correlation coefficient. Triacylglycerol (TAG), 36:4- and 36:5-monogalactosyldiacylglycerol, 34:2- and 36:2-digalactosyldiacylglycerol, 34:1-, 36:1- and 36:6-phosphatidylcholine, and 34:1-phosphatidylethanolamine increased by the stress and immediately decreased during recovery. The relative amount of one TAG species (54:9-TAG) containing alpha-linolenic acid (18:3) increased under heat stress. Microarray data revealed candidate genes responsible for the observed metabolic changes. The genes involved in the eukaryotic glycerolipid synthesis pathway, TAG synthesis/degradation, and lipid turnover were suggested to be induced by heat stress. Lipidomic analysis isolated a T-DNA-inserted mutant line, which showed different accumulation pattern of the lipid species with unsaturated fatty acids under heat stress. These results suggest that heat stress in *Arabidopsis* leaves induces an increase in TAG levels, which likely functions as an intermediate of lipid turnover, and results in a decrease in membrane polyunsaturated fatty acids.

**Poster #: 315**

**Abstract #: 2091**

**Abstract Title:** Metabolic reprogramming in *Sorghum bicolor* in response to *Colletotrichum sublineolum* infection.

**Authors:** Fidele Tugizimana, Ian Dubery,

**Presenting Author Affiliation:** University of Johannesburg

**Abstract Submission:**

Metabolic reprogramming in *Sorghum bicolor* in response to *Colletotrichum sublineolum* infection

Ian Dubery, Fidele Tugizimana, Paul Steenkamp, Arnaud Djami-Tchatchou, Lizelle Piater

Department of Biochemistry, University of Johannesburg, South Africa

Metabolomics is a powerful tool to interrogate cellular biochemistry, investigating metabolism and its reciprocal crosstalk with cellular signalling and regulation. In this study LC-MS-based metabolomics was employed to investigate the metabolic reprogramming reflecting biochemical processes in *Sorghum bicolor* responding to *Colletotrichum sublineolum*. Understanding the molecular mechanisms underlying host plant responses to pathogen infection is essential in controlling diseases associated with reduction of crop yield.

Three cultivars were selected and seedlings were spray-infected with fungal spore suspensions (106 spores/ml). Infection was monitored from 1-9 days post inoculation (d.p.i). Non-treated plants were used as negative controls. Intracellular metabolites from these plants were methanol-extracted and analysed by UHPLC-HDMS. Data analyses were performed using PCA and OPLS-DA. The extracted features were annotated, and metabolic pathway analyses aided in mapping the molecular landscape for biological interpretation. Furthermore, expression levels of some defence-related genes (PRs, PPO, PAL and F3H) were analysed.

The PC analyses of Pareto-scaled data of samples showed time-point (1-9 d.p.i.) clusters and cultivar-related groupings, thus indicating differentiated metabolic profiles over time. PCA results showed that the cultivars responded differently, also confirmed by gene expression results: early, bi-phasic response and amplitude of the response. This defence response to the *C. sublineolum* infection was reflected by metabolic reprogramming, characterised by changes in phenylpropanoid-, flavonoid- and anthocyanin biosynthesis. These metabolic changes involved the de novo biosynthesis of 3-deoxyanthocyanidin phytoalexins, apigeninidin and luteolinidin in all three cultivars. These observations were confirmed by the gene expression results that showed upregulation of PAL and flavonoid 3'-hydroxylase (F3H). The results indicate that the sorghum response to fungal infection is cultivar-dependent, involving dynamic cellular reprogramming of different metabolic pathways.

**Poster #: 316**

**Abstract #: 2123**

**Abstract Title:** Regulation of respiratory metabolism in response to flooding stress as revealed by <sup>13</sup>C-stable isotope redistribution

**Authors:** CARLA ANTONIO, Carola Pöpke, Marcio Rocha, Houssein Diab, Anis Limami, Toshihiro Obata, Alisdair Fernie, Joost van Dongen,

**Presenting Author Affiliation:** Plant Metabolomics Laboratory (ITQB NOVA)

**Abstract Submission:**

Based on enzyme activity assays and metabolic responses to waterlogging of the legume *Lotus japonicus*, it was previously suggested that, during hypoxia, the tricarboxylic acid cycle switches to a noncyclic operation mode. Hypotheses were postulated to explain the alternative metabolic pathways involved, but as yet, a direct analysis of the relative redistribution of label through the corresponding pathways was not made. Here, we describe the use of stable isotope-labeling experiments for studying metabolism under hypoxia using wild-type roots of the crop legume soybean (*Glycine max*).

[<sup>13</sup>C]Pyruvate labeling was performed to compare metabolism through the tricarboxylic acid cycle, fermentation, alanine metabolism, and the GABA shunt, while [<sup>13</sup>C]glutamate labeling was performed to address the metabolism via glutamate to succinate. Following these labelings, the time course for the redistribution of the <sup>13</sup>C label throughout the metabolic network was evaluated with gas chromatography time of flight mass spectrometry (GC-TOF-MS). Our combined labeling data suggest the inhibition of the tricarboxylic acid cycle enzyme succinate dehydrogenase, also known as complex II of the mitochondrial electron transport chain, providing support for the bifurcation of the cycle and the down-regulation of the rate of respiration measured during hypoxic stress. Moreover, up-regulation of the GABA shunt and alanine metabolism explained the accumulation of succinate and alanine during hypoxia.

**Poster #: 317**

**Abstract #: 2170**

**Abstract Title:** Metabolomics-based genome wide association study combining with network analysis provides insights into Arabidopsis secondary metabolism

**Authors:** Si Wu, Lothar Willmitzer,

**Presenting Author Affiliation:** Max Planck Institute of Molecular Plant Physiology

**Abstract Submission:**

Plant secondary metabolism is a coordinated, complex and flexible network of processes tightly regulated at the genetic level, allowing plants to interact with the environment properly. The genetic basis of this network can be explored by analyzing the metabolic compositions of the system. Here, we report an integrative approach combining genetic mapping and transcript-metabolite correlation networks to identify associations between transcripts and secondary metabolites in Arabidopsis. Genome-wide association study (GWAS) was used to unravel the genetic architecture of each metabolite trait and to find metabolic quantitative trait loci (mQTL). Additionally, correlation network analysis based on time-course stress experiments provided co-regulation information between gene expression and metabolite level under abiotic stresses. With this integrative approach, we successfully found strong associations between well-characterized secondary metabolites (e.g. glucosinolates and flavonoids) and the genes involved in the relevant pathways, serving as positive examples to validate the feasibility of our combined approach. Subsequently, we applied this approach to the realm of unknown secondary metabolites. One promising association involved in phenylpropanoid metabolism is extensively discussed in the report in order to illustrate the pipeline we employed to identify unknown secondary metabolites (using isotope labeling and fragmentation analysis) and the causal genes. In total, 142 candidate associations between structural genes and secondary metabolites have been selected from the overlap between the GWAS and the correlation network analysis, as well as from the GWAS per se and the network analysis per se. In conclusion, our results demonstrate that the integrative strategy combining GWAS and network analysis offers a valuable tool for the systematic study of Arabidopsis secondary metabolism.

**Poster #: 318**

**Abstract #: 2052**

**Abstract Title:** Interaction between pathogenic bacteria and higher organisms -MALDI-Imaging based study reveals novel secondary metabolites

**Authors:** Matthias Szesny, Dirk Wunderlich, Jens Fuchser, Stephanie Grond, Florian Zubeil, Dorothee Weisbrod,

**Presenting Author Affiliation:** Bruker Daltonik

**Abstract Submission:**

Over the recent years MALDI-Imaging analysis has gained high interest and the ever increasing number of publication demonstrates its wide applicability.

In the field of Small Molecule Imaging TOF analyzers suffer from interferences from matrix peaks and, thus, specificity can only be achieved with MS/MS experiments. This draw-back can be overcome by using a medium pressure MALDI source and a high resolution mass detector, such as a Fourier Transform Ion Cyclotron Mass spectrometer (FTICR MS). This technique provides sufficient mass resolution and mass accuracy to enable to detect and distinguish thousands of mass signals in a single mass spectrum.

The aim of this study is to employ MALDI Imaging to gain insights about interaction between microorganisms and higher organisms. The infection of potatoes with phytopathogenic bacteria of the *Streptomyces* genus is the cause for substantial loss in crop harvest. Thus the understanding of this interaction might lead to more efficient treatments to protect the crop. The organic molecules involved in the pathogenicity process can be measured by mass spectrometric methods and depicted as images.

Here we present results from an incubation experiment of a *Streptomyces bottropensis* on potato slices. During the analysis of the imaging data new signals in the MS were detected which gave rise to a recently identified necrosis factor and further new metabolites emerging from *S. bottropensis* under these cultivation conditions. By using the intrinsic mass accuracy and ultrahigh mass resolving power of the FTICR instrument we could unambiguously identify the molecular formulae of these new compounds directly from tissue. Key to this data assignment is the possibility to analyze the Isotopic Fine Structure.

Comparing the distributions through a vertical slice of a potato the new compounds are co-located with known Ionomycins.

**Poster #: 319**

**Abstract #: 2160**

**Abstract Title:** A multi-omic approach to reveal the effect of low-level gamma radiation in rice seeds

**Authors:** Hayashi G, Junko S, Kubo A, Imanaka T, Agrawal GK, Shioda S, Fukumoto M, Oros G, Rakwal R, Deepak SA, Seetaramanjaneyulu Gundimeda, Upendra Simha, Arunkumar Padmanaban,

**Presenting Author Affiliation:** Tohoku University

**Abstract Submission:**

: In this study, we performed multi-omic study to investigate the effects of gamma radiation on seeds of rice plants (cv. Koshihikari) grown in radionuclide contaminated soil at Iitate farm located in Iitate village consequent to the nuclear power plant disaster of March 2011, Fukushima prefecture. Rice plants (*Oryza sativa* L. cultivar Koshihikari) cultivated in radionuclide-contaminated soil from a paddy field in IITF (Iitate village in Fukushima prefecture) were collected and stored at ambient temperature. Seeds from rice plants (cv. Koshihikari) grown in clean soil served as control. The gene expression analysis was conducted using rice 4 x 44 k microarrays. The metabolite analysis was done by using LC/MS and GC/MS. The gene expression and metabolomics datasets were combined by creating multi-omics experiment in GeneSpring / MPP / Pathway Architect 13.1 which enables correlation and pathway analysis for different types of omics data. Gene expression microarray analysis revealed 1891 and 440 genes as gamma ray inducible and repressible genes, respectively ( $P < 0.05$ ). The expression values obtained from gene expression microarrays were on par to the values of qRT-PCR. A total of 383 metabolites were identified and fifty differential metabolites were identified using the MPP module of GeneSpring. The combined multi-omics analysis revealed modulation of several metabolic and defense pathways related to stress response of plants. Two metabolites, linolenic acid and 12-OPDA, along with the ACX gene in alpha linolenic acid metabolism, were up-regulated. This pathway is involved in the production of the hormone jasmonic acid, which is involved in stress responses of plants. Our results suggest that the rice plants grown up in radionuclide contaminated soils form seeds with elevated capability to defend well by eliciting appropriate stress responses

**Poster #: 321**

**Abstract #:** 2165

**Abstract Title:** Glycerophosphocholine profiling of the coral symbiotic algae in response to slight copper contamination

**Authors:** Chuan-Ho Tang, Shu-Han Shi, Shu-Hui Lee, Wei-Hsien Wang,

**Presenting Author Affiliation:** National Museum of Marine Biology and Aquarium

**Abstract Submission:**

Slightly copper pollution revealed in coral reefs as a result of adopting copper as active agent for antifouling of boats. Whereas the chronic effect on the coral is poorly known, especially in the symbiotic algae. To gain insight into the impact on the coral health, the coral *Seriatopora caliendrum* was exposed to copper levels above the background by 1-5  $\mu\text{g Cu/L}$  within 96-h duration.

Glycerophosphocholine profiling in the symbiotic algae in response to copper exposure was performed. The result shows that the alteration of lipid profile accompanying several physiological changes was induced in the symbiotic algae. Based on the biochemical and biophysical properties of these changed lipids, the relation between the accommodation of cellular membrane to copper-induced oxidative situation and physiological outcome is deduced in the symbiotic algae.



**Poster #: 322**

**Abstract #: 2329**

**Abstract Title:** Mountain beetle pine infestation: Acetone extractives polar fraction of Lodge pole (pinus contorta) pine

**Authors:** Roderquita Moore, Michael Leitch, Erick Arellano-Ruiz, Doreen Mann,

**Presenting Author Affiliation:** USDA-Forest Service R&D

**Abstract Submission:**

Mountain pine beetle infestation of lodge pole pine is a major concern. The mortality of the tree once attacked is within one year. The ultimate focus is to characterize high value chemicals from underutilized wood. This research is apart of the biorefinery initiative to produce fuels, power, and chemicals from biomass. Lodgepole extractives were separated and the acetone polar fraction was characterize by GC-MS and GCxGC TOF. The chemicals were co-eluting needing a more resolving power to investigate the chemicals in the polar fractionation. Silivchemicals which are chemicals derived from trees are used in cosmetic, paints and oils. The sap and heartwoods of the polar control and the infected were characterize to compare the differences in the chemical composition. The polar component carries the toxic chemicals which can be used for drug development. From this study we should be able to show differences in the chemicals metabolized due to the attack. The instrumentation being utilize also will show the trace chemicals ordinarily would not be detected in the GC-MS.

**Poster #: 323**

**Abstract #: 2460**

**Abstract Title:** Cloud-based analysis of complex sample systems for outlier and trend analysis in the context of water monitoring

**Authors:** Ralf Tautenhahn, Tim Stratton, Dipankar Ghosh,

**Presenting Author Affiliation:** Thermo Fisher Scientific

**Abstract Submission:**

Many analytical challenges attempt to answer a common set of questions – What has or is changing in my system? Is this new sample like the previous ones? These are often asked in specific contexts such as authenticity of a food product or the change in the profile of contamination in water year over year due to new emerging contaminants. Here we introduce a cloud-based system which enables these kinds of problem solving analyses. It consists of a means of processing these potentially large datasets, creation of a representative profile database of components, and hypothesis testing tools to detect differences and trends within the data. Water samples analyzed by a common LC methodology were uploaded to the platform for analysis within the Thermo Fisher Cloud. The samples represented study of a watershed over many months of routine monitoring and the initial step of the processing was to identify several thousand compounds consistently observed across the dataset. Statistics for each component, representing the average level and range observed for each component allowed for a profile to be developed over time. This growing database was also then used to attempt to answer two questions – What is different in an acute time scale and what major trends in the profile could be identified over time. For the first, a new dataset for a recent time point was added to the platform and a comparison performed to the constructed database. Compounds were highlighted that were present at levels statistically significantly different than the historical data. For the second question, an analysis of the data for trends was performed considering both seasonal cycle and trend over time. Components which were identified as potentially trending upward were subjected to specific scrutiny for identification.

**Poster #: 324**

**Abstract #: 2084**

**Abstract Title:** Meta-metabolomics, what did you expect ?

**Authors:** Cyril JOUSSE,

**Presenting Author Affiliation:**

**Abstract Submission:**

Currently, metabolomics use standardized procedures and workflows to ensure biomarkers' relevance and the possibility to merge data with more mature "omics" (genomics, transcriptomics, proteomics). The outputs are promising, revealing scientific and economic opportunities for personalized medicine, micro-scaled agronomy, up scaled biotechnologies, survey of global disturbances on animals, plants and microorganisms, ultimately environment.

Nevertheless, taking into account the influences of each individual in an ecosystem is the challenge for making it a comprehensive figure. We know now that: a human body is a superorganism, comprising many exogenous genes and exposed to xenobiotics plant physiology is strongly impacted by soil rhizoflora and at the same time by infections coming from the air. We also know, for a long time, that microorganisms are everywhere including clouds. They compete for space and nutrients. From biofilms to cloud droplets, they can change our lifestyle, impact health and fitness and modify communities' evolution.

An exhaustive evaluation of metabolic interplays between individuals (animals, plants or microbes) in medicine, agronomy or environment research looks accessible and, sooner or later, mandatory. For achieving this properly, the design of relevant meta-metabolomics experiments is needed. However, we are not ready yet to perform such projects with good chance of success. We need tools, protocols we need to gather knowledge and skills, to maintain the richness of the metabolic information from the field to bench scale, and to computer. First assays are well-known: create model (simplified / labeled) consortia develop wiser NMR and MS-based tools deal with multi-scaled information (multiblocks) synthesize the quintessence (relevance) into in silico ecosystems.

To conclude: meta-metabolomics is the opportunity to embrace the entire information available in every biological context of study. And if we want to succeed, international efforts have to be proposed, granted and made available to everybody (from experimental design to results).

**Poster #: 325**

**Abstract #: 2433**

**Abstract Title:** Ecometabolomics – Understanding biodiversity on a chemical level

**Authors:** Alexander Weinhold, Onno Calf, Michael Singer, Camille Parmesan, Nicole van Dam,

**Presenting Author Affiliation:** iDiv - German Centre for Integrative Biodiversity

**Abstract Submission:**

Plants interact in many different ways with their environment. Many of these interactions are mediated by plant metabolites, which makes their metabolome an important ecological trait. The study of the plant metabolome, or any other organism, in its natural environment is called ecometabolomics. Especially, if we want to understand which processes play a role at the emergence and maintenance of biodiversity in natural environments ecometabolomic studies are of great importance.

Currently, most metabolomics studies are focusing on single model systems or crops. This has led to great insights in diversity of plant metabolomes, including intra- and interspecific variation and phenotypic plasticity in response to biotic and abiotic factors. Dealing with plasticity of plant responses is one of the greatest challenges in ecometabolomics studies. However, if we aim to understand how plant metabolomes are influencing biodiversity processes in natural communities, we have to find ways to manage those challenges.

Here we highlight some of our recent metabolomics analyses on non-model natural plant species. Bittersweet nightshade (*Solanum dulcamara*) and pinewoods lousewort (*Pedicularis semibarbata*) shoot samples were analysed using an UHPLC-QToFMS based approach. The analysis of *S. dulcamara* revealed large differences in metabolomes within and between populations, which may be related to differences in slug resistance. *P. semibarbata* exhibited a high variability in metabolite profiles between different plant populations that might be reflected in different feeding and oviposition preferences of the checkerspot butterfly *Euphydryas editha*.

These results obtained by taking an ecometabolomics approach are the first steps in gaining more knowledge on how the plant metabolome is influenced by populations and how this may affect the next trophic level. They also illustrate that ecometabolomics studies are an important tool to broaden our understanding of how biodiversity and plants metabolomes are connected.

**Poster #: 326**

**Abstract #: 2566**

**Abstract Title:** Environmental Metabolomics Using a Sentinel Species: Methodological Considerations

**Authors:** Christina Jones, John Bowden, Russell Day, Tracey Schock,

**Presenting Author Affiliation:** National Institute of Standards and Technology

**Abstract Submission:**

Environmental metabolomics is an emerging strategy that exploits organismal metabolic changes in an effort to better understand how the environment can affect health. The concept of environmental metabolomics extends further, with parallels to human health studies, to not only provide new insight into response mechanisms in relation to environmental stress, but also as an approach to search for critical biomarkers for environmental disease as well as ascertain etiology of disease and potential health consequences of contaminant exposure.

Mass spectrometry (MS)-based environmental metabolomics at Hollings Marine Laboratory has begun to optimize targeted GC- and LC-MS methods to investigate central carbon, amino acid, and nucleic acid metabolism in pivotal environmentally-related sentinel species, such as coral. Corals are a prime sentinel species owing to the fact that they serve as proxies for ocean surface temperature and salinity, in addition to aiding in understanding the impact of ocean acidification on marine life. Specifically, optimization of the targeted-MS metabolomics methods is aimed at determining and establishing the metabolome of coral skeleton and coenosarc to be used for future health assessments. In conjunction, sample storage, handling, and processing protocols that ensure metabolome stability and allow for the excision of coral coenosarc from the skeletal structure, while preserving the metabolome of both, were also evaluated. Future work will focus on utilizing the developed and optimized targeted-MS metabolome methods to molecularly characterize corals suffering from growth anomalies or disease.

**Poster #: 327**

**Abstract #: 2386**

**Abstract Title:** Stereoselective regulation of metabolism of tobacco cells under salinity stress by means of ornithine enantiomers

**Authors:** Morteza Gholami,

**Presenting Author Affiliation:** Department of Chemistry, Golestan University, Gorg

**Abstract Submission:**

The urea cycle is a central metabolic pathway in plant cells functioning in a wide range of nitrogen (N) and carbon (C) metabolism. In this research, a stereospecific regulation of the urea cycle by exogenous L- and D-Orn was investigated to ascertain the role of enantiomers (D and L) on the metabolism of tobacco cells under normal and stress conditions. Results 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 of homoserine or monosaccharides biosynthesis which led to the alleviation of salinity effects in treated 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 cell growth enhancement, alleviation of stress effects and selective regulation of certain metabolic pathways can be potentially performed by means of exogenous D-Orn, what has not been observed before for any other D-AAs in the plant cells.

**Poster #: 328**

**Abstract #: 2457**

**Abstract Title:** Influence of enhanced CO<sub>2</sub> concentration and light/dark cycles on growth, biochemical composition and metabolic profile of *Chlorella vulgaris* (CCAP 211/21A)

**Authors:** Rahul Kapoore, Maria Huete-Ortega, Katarzyna Okurowska, Seetharaman Vaidyanathan,

**Presenting Author Affiliation:** The University of Sheffield

**Abstract Submission:**

Algae are one of the most important bio-factories on earth based on their photosynthesis/CO<sub>2</sub> fixation capacity and supposed to be biofuels of the third generation. Not only do these organisms fix carbon dioxide, but they also have the potential to be used for the production of inexpensive bulk chemicals because the major inputs into the system (light and CO<sub>2</sub>) are essentially free. To harness this potential through metabolic engineering, a deeper understanding of photosynthetic metabolism is required first. As metabolites are the first to react to stressors, it will be advantageous to carry out the evaluation of stress induced effects in microalgae in combination with multivariate tools. Here we studied the influence of enhanced CO<sub>2</sub> concentration and light/dark cycles on growth, biochemical composition and metabolic profile of *Chlorella vulgaris* (CCAP 211/21A). *Chlorella vulgaris* was cultured under two different CO<sub>2</sub> concentrations (0.04% (air) and 1% at 1 vvm) under light/dark (16/8h) cycles. Samples were harvested every day at 4h into light and dark cycle until stationary phase (day 14) is reached. The samples were then analysed for their biochemical composition using spectroscopic methods, total fatty acids (FAME) by GC-FID and metabolic profile by GC-MS. Relevant changes in the biochemical composition of the cells including carbohydrates, lipids, proteins and pigments was observed in response to varied CO<sub>2</sub> concentration. Similarly, the phenotypic changes were tracked by monitoring the profiles of metabolites by GC-MS, which will provide novel insights in understanding the influence of enhanced CO<sub>2</sub> concentration and light/dark cycles in *Chlorella vulgaris*. The results of which will be discussed here.

**Poster #: 329**

**Abstract #: 2001**

**Abstract Title:** Eavesdropping on Marine Microbial Communication: Influence of Quorum Sensing on the *Vibrio campbellii* Metabolome

**Authors:** Gregory Ellis,

**Presenting Author Affiliation:**

**Abstract Submission:**

Gregory A. Ellis 1, Brian Eddie 1, Arnaldo A Torres Padua 2, W. Judson Hervey IV 3, Gary Vora 3, Dagmar H. Leary 3

1 ASEE/NRC/NRL Postdoctoral Fellow 2 University of Puerto Rico Mayagüez, Mayagüez, Puerto Rico 3 Center for Bio/Molecular Science and Engineering (CBMSE), Naval Research Laboratory, Washington, DC 20375, USA

The US Navy faces a significant challenge — ship hull fouling which causes frictional drag leading to annual fuel expenditures of millions of dollars. Marine microorganisms form biofilms which contribute to this fouling. We aim to investigate these marine consortia to understand how they communicate within biofilms and maintain their community, with the ultimate goal of engineering environmentally-friendly anti-fouling methods. Key to investigating this communication will be qualitative and quantitative measures of the small molecule chemistry of these consortia through the use of LCMS-based metabolomics. To develop in-house metabolomics methods, we chose to begin studies with the biofilm-producing, quorum sensing (QS), model bacterium *Vibrio campbellii*. We eavesdropped on the conversation of *V. campbellii* by specifically studying all of the molecular “words” between bacteria in the extracellular space (untargeted “exometabolomics” or “secreted metabolomics”). To determine how the conversation changes dependent on QS, we measured exometabolites of both the wild-type *V. campbellii* as well as a variant lacking the QS transcription factor LuxR (ΔLuxR) at 16 time-points throughout growth. We found that potential signaling molecules indole-3-carboxyaldehyde and several diketopiperazines, as well as lipids from amphi-enterobactin siderophores, were differentially produced in wild-type versus mutant *V. campbellii*. In addition to untargeted studies, we are performing targeted metabolomics studies to quantitate the “volume” of “words” in the *V. campbellii* conversation. The methods developed on this model bacterium will springboard our studies of biofilm consortia, giving insight into community maintenance and targets for disrupting biofilm communication.



**Poster #: 330**

**Abstract #: 2029**

**Abstract Title:** <sup>1</sup>H NMR-based metabolomics reveals that dissolved organic matter alters the toxicity of organic contaminants to *Daphnia magna*.

**Authors:** Vera Kovacevic, André Simpson, Myrna Simpson,

**Presenting Author Affiliation:** University of Toronto

**Abstract Submission:**

The potential deleterious impacts of organic contaminants in aquatic ecosystems are complex because these contaminants may bind to dissolved organic matter (DOM) which may subsequently reduce their bioavailability and toxicity. Polar and ionizable organic contaminants exhibit weak interactions whereas more hydrophobic contaminants may interact strongly with DOM and become less bioavailable. To test this hypothesis, we used <sup>1</sup>H nuclear magnetic resonance (NMR)-based metabolomics to investigate the sub-lethal acute toxicity to *Daphnia magna* of four individual contaminants with varying hydrophobicity. Daphnids were exposed for 48 hours to sub-lethal concentrations of ethynyl estradiol (EE2 1 mg/L), the relatively more polar compounds carbamazepine (8 mg/L) and imidacloprid (1.5 mg/L), or perfluorooctane sulfonate (PFOS 30 mg/L) in the absence and presence of Suwannee River DOM (0 and 5 mg organic carbon/L). EE2 exposure showed evidence of oxidative stress with elevated levels of most amino acids and the addition of DOM entirely mitigated this metabolic response. Carbamazepine exposure resulted in the decrease in several metabolites but these metabolites did not change in the presence of DOM. The metabolic response to imidacloprid exposure did not differ with DOM. However, PFOS exposure resulted in a more significant metabolomic response with DOM suggesting that DOM enhanced the bioavailability of PFOS. Since EE2 toxicity was reduced by DOM more than the other contaminants studied, subsequent exposure experiments with *D. magna* were carried out with varying levels of Suwannee River DOM (0, 1, 2, 3 and 4 mg organic carbon/L). The higher concentrations of DOM (3 and 4 mg organic carbon/L) resulted in a greater reduction in the metabolic response to EE2 exposure. <sup>1</sup>H NMR-based metabolomics revealed that DOM can greatly alter the toxicity of organic chemicals. The differences in the metabolic shifts are likely related to the binding affinities of these contaminants to DOM, as well as DOM concentration.

**Poster #: 331**

**Abstract #: 2417**

**Abstract Title:** A multiscale modeling approach including metabolomics reveals the differential impact of dairy fats on the development of atherosclerosis in hamsters

**Authors:** martin jean-charles, daniel dalemans, bernadette delplanque,

**Presenting Author Affiliation:** amu

**Abstract Submission:**

Various types of experimental dairy fats were tested in the hyperlipemic hamster to assess their effectiveness in modulating atherosclerosis.

Multi-platform analysis coupling omics approaches and conventional analyses were conducted to explain the severity of atherosclerotic lesions: high resolution plasma and urine LCMS metabolomics, high throughput fatty acid profiling in plasma and liver lipids, blood chemistry, and expression of 50 genes in the liver and blood associated to metabolic disorders. Overall 1666 variables described the biological status of each Hamster.

The biological variables related to the disease were selected using PLS methods. Their relationships were examined in a high density interaction network. Examination of the network sub-regions and of the heterogeneous variables ontology allowed to assemble them into 10 biological functional sets or "biological modules": amino acid metabolism, regulation of metabolism and lipid transport, mitochondrial function, endogenous fatty acids, dairy fat derivatives, metabolism of vitamin E, blood cholesterol related, inflammation, hemostasis, unclassified/unknown. Each module was scored using a multi-block analysis. These scores were assembled into a PLS regression equation, to predict over 80% of the observed atherosclerosis. In this equation, the weight of the module "regul. Lipid Metabol. Transport" was essential to explain atherosclerosis. Partial correlations network analysis of the biological scores again stressed the importance of this module on the development of atherosclerosis, due to its geocentrality and its direct link with the pathological score. Furthermore, our results showed that the activation of certain biological modules were linked to the fat quality, while others were not. Thus, "metabolism of vitamin E" was the best contributor to the atheroprotective effect of the modified fat inducing less vascular lesions.

In conclusion, our modeling approach coupling metabolomics to other biological approaches helped identify and quantify the contribution of complex factors in atherogenesis development in each nutritional setting.

**Poster #: 332**

**Abstract #: 2482**

**Abstract Title:** Ecometabolomics for Biodiversity: Tapping into chemical communication in the wild

**Authors:** Nicole van Dam, Alexander Weinhold, Katharina Grosser, Stan Harpole,

**Presenting Author Affiliation:** iDiv – German Centre for Integrative Biodiversity

**Abstract Submission:**

With this poster I introduce the new Ecometabolomics platform at the German Centre for Integrative Biodiversity Research (iDiv) Halle-Jena-Leipzig. The aim of this facility is to become an international centre to train and support ecologists and biodiversity researchers to properly implement metabolomics analyses in their research projects. Currently our facility contains a Bruker Impact HD LC-qToF-MS (secondary metabolites), a Bruker EvoQ LC-QqQ (hormones, targeted analyses), a SCIION GC-QqQ (Bruker) equipped with a Markes Thermodesorber Unity 2 (volatiles), and a Thermo Scientific/Dionex HPLC-PDA (glucosinolates). In addition we have analytical platforms that are more commonly used in ecological research such as a C/N analyser (Elementar) and a MPA FT-NIR (Bruker). The latter allow for more global assessments of plant chemical composition. We closely collaborate with iDiv members and associated centres on chemical-analytical methods (MPI-CE, Meredith Schuman, Ian Baldwin), data workflows (IPB-Halle, Steffen Neumann, Dierk Scheel), bio-informatics for MS/MS spectral identification (Michael Stifel Centrum, FSU Jena, Sebastian Böcker), data management (BDU-iDiv, Birgitta König-Ries, Jitendra Gaikwad), multivariate statistics and data visualisation (BIU-iDiv, Yvonne Poeschl MLU, Ivo Grosse). We aim to promote eco-metabolomics within iDiv and beyond by organising lectures and training seminars on metabolomics applications in biodiversity research as well as proper data processing, analysis and archiving. Moreover, we are welcoming partners to develop externally funded joint research projects. The research projects of Molecular Interaction Ecology at iDiv focus, amongst others, on identifying novel metabolites involved in communication in soil foodwebs, specifically on exudates and root volatiles. For this application, we are currently designing novel set-ups for sampling belowground metabolites, e.g. root volatiles, root exudates, and bacterial volatiles, under non-sterile conditions.

**Poster #: 333**

**Abstract #: 2530**

**Abstract Title:** Untargeted lipidomic study of UV radiation effects in human skin cells

**Authors:** Núria Dalmau,

**Presenting Author Affiliation:** IDAEA-CSIC

**Abstract Submission:**

UV radiation present in sunlight and in artificial light source is an environmental human carcinogen. Epidemiological studies suggest that chronic exposure to UV solar radiation is responsible for skin tumour development via gene mutations and immunosuppression. Solar UV radiation is composed by ultraviolet B radiation (290-320 nm), which is responsible for direct DNA damage, and ultraviolet A radiation (320-400 nm), which causes indirect damage through the generation of reactive oxygen species.

In this work, an untargeted lipidomic analysis has been performed in order to investigate which are the important lipids involved in the effects of UV solar radiation in melanocytes, keratinocytes and melanoma cells. This study was carried out using a solar simulation unit to expose these cells to different intensities of irradiation.

Experimentally, cells were exposed under acute conditions, which were optimized using cell viability tests. Then, a lipidomics study was carried out by UHPLC-TOF-MS. Multivariate data analysis (PCA, PLS-DA and MCR-ALS) methods were used to reveal the changes in the concentrations of the main lipids due to UV solar radiation effects in the investigated cell models. Once these lipids were identified, they were used to decipher the biologic pathways involved in UV radiation cell damage and to obtain a characteristic lipid signature of these UV radiation effects.

**Poster #: 334**

**Abstract #: 2072**

**Abstract Title:** Metabolomic Study of Environmental Exposure: Effects of Perfluoroalkyl Substances and Phthalates on Children

**Authors:** Zhi-Yi Du, Guang-Wen Lien, Pau-Chung Chen, Ching-Yu Lin,

**Presenting Author Affiliation:** Institute of Environmental Health, College of Publ

**Abstract Submission:**

Perfluoroalkyl substances (PFASs) and phthalates are both commonly used in industrial applications and consumer products, and therefore they can be detected in the human samples widely. Recent epidemiologic studies have been focused on some health effects of PFAS and phthalate exposure in the human. However, these epidemiologic surveys still cannot provide complete and clear association to link possible mechanisms of these chemicals and their adverse health effects. The purpose of this study is to understand possible mechanisms of environmental exposure to PFASs and phthalates in causing adverse health effects in children, who can provide a trajectory for significant effects in adulthood, by using metabolomic approach. 290 Taiwanese children (8-10 years) exposed to background levels of PFASs and phthalates were included in this study. Thirteen PFASs and twelve phthalate metabolites were analyzed in their biofluids by high performance liquid chromatography/ tandem mass spectrometry. Proton nuclear magnetic resonance spectrometry combined with multivariate statistical methods and multiple linear regression was applied to examine serum particular metabolic patterns in children exposed to different levels of PFASs and phthalates. Moreover, questionnaire data were collected to associate with the changes of metabolome and exposure levels of these chemicals. In our results, different metabolic patterns were discovered in children exposed to different levels of PFUnDA (perfluoro-n-undecanoic acid), PFTrDA (perfluoro-n-tridecanoic acid), MiBP (mono-isobutyl phthalate) andSDEHP (Di(2-ethylhexyl) phthalate). In addition, the metabolomes of children's serum were associated with their residential regions and their body mass indexes. Metabolites associated with PFAS and phthalate exposures and others were identified. This study shows that metabolomics is a powerful approach to identify metabolic perturbation caused by environmental exposure and to suggest possible modes of action of these chemicals and their possible adverse health effects.

**Poster #: 335**

**Abstract #: 2145**

**Abstract Title:** Metabolomic Unveiling of Distinctive Metabolism between Semi-wild and Wild Soybean

**Authors:** Dae-Yong Yun, Young-Gyu Kang, Myoyeon Kim, Jun Seong Park, John Hwan Lee, Young-Shick HONG,

**Presenting Author Affiliation:** Chonnam National University

**Abstract Submission:**

Soybeans are an important crop for agriculture and food, leading to increase of a range of its application. In order to better understand the physiology of different soybean cultivars with chemical compositions, we investigated the metabolic evolution and cultivar-dependent metabolite variation in the leaves of domesticated (*G. max*) and semi-wild (*G. gracilis*) soybean, through a <sup>1</sup>H NMR-based metabolomics approach, as they grew from V1 to R7 stages. The levels of primary metabolites, such as sucrose, amino acids, organic acids and fatty acids, were decreased both in the *G. gracilis* and *G. max* leaves. However, the secondary metabolites, such as pinitol, rutin and polyphenols, were increased, while synthesis of glucose was elevated as the leaves grew. Comparing metabolite variations between *G. gracilis* and *G. max*, it was noteworthy that rutin and its precursor, quercetin-3-O-glucoside, were found only in *G. gracilis* but not in *G. max*. Furthermore, levels of pinitol, proline,  $\gamma$ -alanine and acetic acid, a metabolite related to adaptation toward environmental stress, were different between the two soybean cultivars. These results highlight their distinct metabolism for adaptation to environmental conditions and their intrinsic metabolic phenotype. This study therefore provides important information on the metabolic evolution and cultivar-dependent metabolites of soybean leaves for better understanding of plant physiology to develop soy-based products.

**Poster #: 337**

**Abstract #: 2494**

**Abstract Title:** UNTARGETED LCxLC-HRMS APPLIED TO THE METABOLOMIC STUDY OF CIRCADIAN RHYTHM IN JAPANESE RICE

**Authors:** Meritxell Navarro-Reig, Joaquim Jaumot, Gabriel Vivo-Truyols, Peter Schoenmakers, Romà Tauler,

**Presenting Author Affiliation:** IDAEA-CSIC

**Abstract Submission:**

Over the past years, the complexity of analytical samples has increased in many applied research fields, such as environmental, health and food analysis. Liquid chromatography is often the analytical method selected for the analysis of these samples, due to its ability to resolve complex natural mixtures. However, one-dimensional liquid chromatography is often not able to separate all the constituents present in these highly complex samples. Comprehensive two-dimensional liquid chromatography (LCxLC) is a very powerful alternative to their complete resolution.

Metabolism in plants is rolled by the circadian rhythm, which adapts the different biological functions to the day period. This work aims at identifying the principal metabolites involved in the Japanese-rice circadian clock under a drought stress and to analyse their dynamic changes over a 24-hour period. For this purpose, an untargeted LCxLC-HRMS method has been developed. In the first chromatographic dimension, a HILIC TSK gel Amide-80 column (250×2.0 mm 5 µm) was employed. Using a two-way, 10-port valve with two switching loops, fractions from the first chromatographic dimension were introduced in the second chromatographic dimension consisting of a Kinetex C18 (50×2.1 mm 1.7 µm) column. The complexity of the analysed rice samples and the vast size of the LCxLC-HRMS data set made the analysis and resolution of the complete data set very challenging.

Resolution of the LCxLC elution profiles and of the MS spectra of each detected metabolite in every sample was performed using multivariate curve resolution–alternating least squares (MCR-ALS). From these MCR-ALS results, the changes in metabolite concentrations along the circadian experiment and the identification of the different metabolites using their MCR-ALS resolved HRMS spectra and online library were established.

The proposed strategy allowed identifying more than 100 statistically significant metabolites, which were then used to highlight the most probable metabolic pathways involved in the studied circadian rhythm.

**Poster #: 338**

**Abstract #: 2261**

**Abstract Title:** Zebrafish response to low concentration perfluorinated compounds PFOA and PFOS: Metabolomic perspective

**Authors:** Carl Angelo Medriano, Ryan De Sotto, Sungpyo Kim, Youngja Park,

**Presenting Author Affiliation:** Korea University, College of Pharmacy

**Abstract Submission:**

Perfluorooctanoic acid(PFOA) and Perfluorooctane sulfonate (PFOS) are persistent organic compounds that are consistently being detected in vast number of water bodies, worldwide. Being a known fire retardant, its presence persists when released in the environment. Aside from the water quality, vast environmental species depending on such water bodies would be exposed to such pollutants. However, it is not well known how these emerging contaminants pose a threat to the different water organisms. Therefore, the goal of this study is to analyze the effect of ppb concentration of PFOA and PFOS to zebrafish.

As a model organism, adult zebrafish was used in this study. Three setups (n=10) were dosed with 5ppb of PFOA, three setups were dosed with 5ppb of PFOS, and three undosed setups were used as control. The fish were exposed for 5 days after which each setup was extracted using Bligh and Dyer. The extracts were then analyzed using liquid chromatography-mass spectrometry-based metabolomics. A multivariate statistical analysis was performed to obtain significantly expressed metabolites using a false discovery rate (FDR) multiple testing correction threshold of  $q=0.05$ .

Results show that in PFOA, 27 features were found to be significant compared to the control, while 52 features were found significantly different for PFOS. Hierarchical clustering analysis also showed a clear separation between the two groups for both analysis suggesting that at 5ppb concentration of these compounds, the metabolism of the fish are already affected. Annotation using Metlin database showed similar compounds affected like Phosphatidate (m/z: 701.519 [M+H]<sup>+</sup>), Riboflavin (m/z: 377.138 [M+H]<sup>+</sup>), and Phosphatidylethanolamine (m/z: 684.472 [M+H]<sup>+</sup>) while Pathway analysis using Kyoto Encyclopedia Genes and Genomes suggests various pathways like Glycerophospholipid metabolism, and Cysteine and methionine metabolism possible affected by the low concentration of PFOA and PFOS.



**Poster #: 339**

**Abstract #: 2521**

**Abstract Title:** Community Metabolomics for Analysing Stress Responses in Environmental Biofilms

**Authors:** Mechthild Schmitt-Jansen,

**Presenting Author Affiliation:** Helmholtz Centre for Environmental Research - UFZ

**Abstract Submission:**

Although metabolomics was applied in an ecological context so far, metabolomics studies on mixed species samples like environmental biofilms are rare. Environmental biofilms are key components for primary production and geo-cycling of world aquatic systems, thus understanding their stress responses is essential for ecosystem functioning. Challenges in community metabolomics derive from the complexity of the metabolomes of the individual species in mixed species assemblages. Additionally, metabolic responses need to be integrated and related to ecological metrics to derive ecologically meaningful information.

Aim of this study was to apply metabolomics to trace stress responses of environmental biofilms, exposed to multiple stressors and relate them to classical community metrics like induced community tolerance: Communities chronically exposed to stress increase their overall tolerance. Stress-induced community tolerance (SICT) could derive from three processes: physiological plasticity of species, succession of species and evolutionary processes. Whereas the relevance of succession for community tolerance is well documented, the role of metabolic responses is less clear.

We exposed environmental biofilms to salt and toxic stress in a mesocosm study. The SICT-approach was used to quantify the community response on an integrated level. Metabolic profiles were derived by shock-freezing biofilm communities and analyzing the polar and apolar fraction by GC-MS after liquid-liquid extraction. Metabolic profiles were analyzed by multivariate statistics and integrated to the MELI (metabolic effect-level index [1]). MELI showed a stress- and time-dependent correlation with community tolerance indicating the relevance of metabolites in stress responses and adaptation processes. Responsive metabolites were identified and related to biochemical pathways.

The presentation will outline how metabolic responses of communities could be emerged in eco(toxico)logical research of communities.

[1] Riedl J., Schreiber R., Otto M., Heilmeier H., Altenburger R., Schmitt-Jansen M. (2015) Metabolic Effect Level Index (MELI) links multivariate metabolic fingerprints to ecotoxicological effect assessment. ES&T, 49(13), 8096-8104.

**Poster #: 340**

**Abstract #: 2114**

**Abstract Title:** Effects of biodiversity on exuded and inner root metabolites in grassland communities

**Authors:** Sophie Dietz, Katharina Herz, Karin Gorzolka, Nadine Strehmel, Ute Jandt, Helge Bruelheide, Dierk Scheel,

**Presenting Author Affiliation:** Leibniz-Institute of Plant Biochemistry, Stress and

**Abstract Submission:**

While much research has been done on aboveground plant parts, there is still a blind spot on belowground root exuded metabolites, called exudates. Such exudates are released by the plant to interact with their surrounding environment and to promote the uptake of nutrients. This cooperative project between ecology and mass spectrometry aims to fill this gap by the application of untargeted metabolite profiling on exudates and inner root metabolites of two common grassland life forms: grasses and forbs. Ten different species of these life forms were grown in the German Biodiversity Exploratories under natural conditions (field) as well as in a phytochamber under controlled conditions (lab) and analysed for their primary and secondary metabolites. The results will serve to investigate if (i) plant exudation behaviour is driven by the plant family or (ii) the species itself. In cooperation with ecologists and microbiologists this research will also help to understand the (iii) functional role of root exudates between plant and microbial biodiversity in the rhizosphere and (iv) whether results from laboratory could be retrieved in a natural environment.

The two different mass spectrometric approaches, GC coupled to EI-Q-MS and RP-UPLC coupled to ESI-Q-TOF-MS, revealed 280 primary metabolites and 1792 secondary metabolites in the field samples. Until now, 84 metabolites were identified with the help of database and MSn. Statistical analysis of both data sets revealed differences between exudation behaviour of both life forms in primary and secondary metabolite profiles. Furthermore, there is evidence that grass species have a relatively common exudate profile whereas forb species exude species specific secondary metabolites. It will be interesting to know their kind of nature and how far they are derived from their metabolites within the root both is in progress.

Work was supported by Deutsche Forschungsgemeinschaft (SCHE 235/16-3)

**Poster #: 341**

**Abstract #: 2448**

**Abstract Title:** The timing of shifts in the transcriptome and proteome in relation to metabolic state during tomato fruit development

**Authors:** Isma Belouah, Thierry Balliau, Camille Bénard, Bertrand Beauvoit, Sophie Colombie, Stéphane Bernillon, Patricia Ballias, Cécile Cabasson, Mickäel Maucourt, Catherine Deborde, Annick Moing, Benoit Biais, Dominique Rolin, Michel Zi

**Presenting Author Affiliation:** INRA -- University of Bordeaux

**Abstract Submission:**

The tomato *Solanum lycopersicum* is a plant model for fruit development. The maturation of the fruit has been dividing into three principal stages of cell division, cell expansion, and maturation according to phenotype, physiology and cellular properties. Each stage is characterised by a unique primary metabolism to advance the continuous development of the fruit. Over the past number of years we have been involved in a Systems Biology study to describe the molecular aspects of metabolism and to create metabolic models describing the course of development. This approach has focused primarily on the control of metabolism through enzyme activity. Noting that most enzymes appear to operate at only a fraction of their  $V_{max}$  measured in vitro, it is intriguing how cells regulate the level of enzyme proteins. Accordingly, we have obtained protein and transcript profiles using label-free quantification and RNA-seq, respectively, to investigate those components governing the amounts of enzymes. We observed that around 30% of both the 2700 detected proteins and 34,000 detected transcripts change significantly over the course of development. Our immediate objective is to provide a qualitative description of transcriptional and proteomic states in tomato fruit in relation to metabolite profiles and enzyme activities obtained over fruit development. According to previous reports, standard principal component analysis (PCA) based on enzyme activities segregated samples according to stage, but with the greatest separation pertaining to the turning/ripening transition. PCA of current data revealed that metabolite or protein levels segregated samples roughly into the three physiological categories of development with protein levels yielding finer resolution of stages. In contrast, transcript profiles split samples primarily between cell division and maturation, with age of the green fruit contributing little to differences among stages. We will present these findings and other statistical analyses integrating the various orders of molecular data we have accumulated.

**Poster #: 342**

**Abstract #: 2518**

**Abstract Title:** Metabolomic analysis of *Saccharomyces cerevisiae* in the presence of imatinib

**Authors:** Hilal Taymaz-Nikerel, Serpil Eraslan, Betul Kirdar,

**Presenting Author Affiliation:** Bogazici University

**Abstract Submission:**

The understanding of the working principles of chemotherapeutic drugs, which are frequently used in the treatment of cancer, is important both for improving existing therapies in addition to finding new drug targets. Imatinib is one of the first chemotherapy drugs that is used successfully as targeted therapy in the treatment of terminal cancers such as gastrointestinal stromal tumors and chronic myeloid leukemia. The known cellular effects of imatinib are inhibition of the activity of the tyrosine kinase enzyme and prevention of the operation of the signal transmission system. However, there is not much information on the selective molecular mechanisms leading to the death of cancer cells and about the reorganization of cell metabolism as a response to imatinib. Quantitative investigation of metabolism helps to understand the changes caused by the drug and thus the affected pathways could be identified. To see the cellular effect of imatinib, *Saccharomyces cerevisiae*, used as a model organism in biomedical and medicinal research, were grown in controlled bioreactors in triplicate in the absence and presence of 600 mg/L imatinib. Samples for metabolome analysis were collected at varying time points. The changes in the intracellular level of metabolites in the glycolytic and amino acid pathways were integrated/compared with the metabolic flux analysis. Results were used to reveal the metabolic effects of imatinib on the yeast cells.

**Poster #: 343**

**Abstract #: 2025**

**Abstract Title:** EXISTENTIAL METABOLOMICS: VISUALIZATION OF GROWTH AND DEVELOPMENT PROCESSES THROUGH METABOLITE PROFILING

**Authors:** Alexey Shavarda, Ekaterina Kotlova, Gregory Pozhvanov, Katerina Sazanova, Svetlana Senik,

**Presenting Author Affiliation:** Komarov Botanical Institute

**Abstract Submission:**

Hardly anyone would dispute the fact that a set of small molecules which compose a living body is fundamentally different from the same molecules extracted from biological object. In the living body, metabolites are in a relationship with each other whereas having been extracted, the only link between the metabolites is the fact that their co-host is in the same flask. The formalization of these relationships under a special concept, called existential, allows a deeper understanding of the very essence of life phenomenon.

We believe that all molecules in a living system must be different even if they have the same structure. From these differences is born the fundamental possibility of systemic communication between molecules. The individuality of the molecules is necessary factor in creating a biological system and ensured by the fact that each of them has its own fate depending on the history of its existence each molecule is born somewhere, had some lifetime and disappears, converted into another. The practical relationships between single molecules manifest themselves in their mutual transformation and in turn, a change in the number of molecules of each metabolite. The network organization of the molecular community leads to the formation of stable hubs – metabolites with a high content. Development of the system, understood as the temporal dynamics of its state, must inevitably be reflected in a shift in the significance of these hubs.

We have conducted metabolite profiling experiments to study growth and development processes in different models at the level of tissues, organs and intact plants. In all cases the representative points in phase space built on the relative abundance of metabolites as coordinates, were visualized using PCA, form specific trends adequate to growth processes. We demonstrate that the correlating structure of the metabolite network corresponds to subsequent states of biological development.

**Poster #: 344**

**Abstract #: 2523**

**Abstract Title:** Metabolite profiling of *Saccharomyces cerevisiae* grown on different carbon sources

**Authors:** Marit Stafsnes, Truls Rasmussen, Per Bruheim,

**Presenting Author Affiliation:** NTNU

**Abstract Submission:**

The yeast *S. cerevisiae* is a versatile organism well known for its usage in traditional biotechnology for beer and wine production, modern biotechnology as host for heterologous production of a variety of biochemical, but also as a model organism in basic biological research. Much is already known about the yeast metabolome and changes due to environmental and genetic perturbations. We wanted to revisit the yeast metabolome with the latest versions of our target quantitative MS methods (2 LC-MS/MS methods and 1 capIC-MS/MS method) covering the primary metabolite groups (amino acids, organic acids, sugar phosphates and other phosphorylated metabolites, complete nucleotide and nucleoside phosphate pools) on a yeast laboratory strain cultivated and sampled under controlled laboratory conditions in bench-top bioreactors. This is important background data for evaluation of stress-responses and genetic perturbations in research projects. The poster will present initial data from batch cultivations on different carbon sources while chemostat experiments with different growth limiting nutrient and different growth rates are currently generated.

**Poster #: 345**

**Abstract #: 2525**

**Abstract Title:** The metabolic response of *Saccharomyces cerevisiae* to linoleic and conjugated linoleic acids

**Authors:** Francesca Casu, Sergey Tumanov, Eliezer Stefanello, Farhana Pinu, Silas Villas-Boas,

**Presenting Author Affiliation:** The University of Auckland

**Abstract Submission:**

During food fermentation *Saccharomyces cerevisiae* metabolises the nutrients present in the substrate and secretes metabolic products. Therefore, its metabolism together with the substrate composition play an important role in determining the characteristics of the final food product. For example, the polyunsaturated fatty acid, linoleic acid, which is present in the grape juice at trace levels, is known to affect the development of aroma compounds and other properties of the wine fermented by *S. cerevisiae*. However, the effect of linoleic acid on the overall cell metabolism is still not clear because only indirect effect on the fermentation products were investigated. Our study aims to unlock the metabolic response of *S. cerevisiae* to linoleic acid using metabolomics. We cultured the cells on glucose supplementing them with linoleic acid and we analysed the intracellular and extracellular profiles to investigate which *S. cerevisiae* pathways are affected by linoleic acid. The transport of linoleic acid into the cell had an impact on the primary carbon metabolism increasing the glucose consumption and the ethanol production. The energetic state of the cell was therefore affected and the glycolytic pathway, the TCA cycle and the amino acid production were up-regulated. Moreover, since *S. cerevisiae* fatty acid profile was altered, we performed an experiment in parallel supplementing the medium with a labelled isotope of linoleic acid to follow its metabolic fate which is metabolised into longer and shorter chain fatty acids.

**Poster #: 346**

**Abstract #: 2262**

**Abstract Title:** Phencyclidine-induced changes in the metabolic activity of the rat brain

**Authors:** Olli Kärkkäinen, Jouni Ihalainen, Katja Savolainen, Kati Hanhineva, Markus Forsberg,

**Presenting Author Affiliation:** University of Eastern Finland

**Abstract Submission:**

Promising preclinical drug candidates often fail in the clinical phase and this has also been the case in development of novel drugs for schizophrenia (SCZ). One key challenge in discovering novel drug candidates for SCZ is to translate SCZ-like symptoms into appropriate preclinical models. Currently, the most commonly used approach is to induce SCZ-like symptoms by phencyclidine (PCP). However, there is a need to understand better the validity of this model.

Here our aim was to study the effects of acute and subchronic PCP exposure to the metabolic activity of the rat brain. Three treatment groups (N = 6 per group) were included in the study: A) saline control, B) acute PCP (3 mg/kg s.c. 2 hours before euthanasia) and C) subchronic PCP (3 mg/kg s.c. for 5 days, twice daily). Brain samples of frontal cortex, striatum and hippocampus were collected and analyzed by non-targeted metabolite profiling with LC-qTOF-MS system using hydrophilic interaction (HILIC) column.

PCP altered brain amino acid metabolism e.g. aspartate levels were lower in PCP-treated animals than those in controls. Furthermore, also differences between acute and subchronic PCP exposure were observed e.g. animals treated with subchronic PCP had lower glutathione disulfide levels than controls. These results may be associated with PCP-induced alterations in amino acid transporters and amino acid metabolizing enzymes, e.g. excitatory amino acid transporter 1 and d-amino acid oxidase. Previously abnormal function of these transporters and enzymes have been associated with schizophrenia in human studies.

In conclusion, PCP altered amino acid metabolism in the rat brain and the observed changes are similar to those previously observed in patients with schizophrenia. Therefore, the present study further supports the face validity of PCP in inducing schizophrenia-like symptoms in rodents.



**Poster #: 347**

**Abstract #: 2598**

**Abstract Title:** A genome-scale map of amino acid metabolism by metabolic profiling of a prototrophic gene deletion collection of *Saccharomyces cerevisiae*

**Authors:** Michael Muelleder, Markus Ralser,

**Presenting Author Affiliation:** The Francis Crick Institute

**Abstract Submission:**

Functional genomic approaches are key in the analysis of cellular processes and gene function prediction. In *Saccharomyces cerevisiae*, the function of 44% of open reading frames is not well understood. Metabolite concentrations are the ultimate cellular response to perturbation and hence provide information on gene function not accessible by other approaches. The aim of this work is the genome-scale functional metabolic profiling of a prototrophic gene deletion collection to map all genetic factors acting on amino acid metabolism.

We established a high-throughput cultivation, sample preparation and analytical HILIC-MS/MS platform and determined free amino acid levels with high accuracy for 4678 of prototrophic mutant strains. Of these, 32% had a significant impact on amino acid metabolism. Among highest scoring signalling and transcriptional regulators we identified the TORC1 pathway and protein complexes for chromatin dynamics. The metabolic signatures were further informative about gene function and allowed to associate 83% of poorly characterised open reading frames to functional categories. We showed the efficiency of this approach by annotating the cytosolic ribosome and identified novel gene functions for TEF4, MCH5 and OPT2 in vesicle fusion and autophagy.

In this study we present a genome-scale dataset for genetic-metabolic interactions focussing on amino acid metabolism. We identify more than 1500 genes important for biosynthetic metabolism, reveal among them the most important genes for regulation on the transcriptional and signalling level and exploit the data as rich resource for functional annotation.

**Poster #: 348**

**Abstract #: 2314**

**Abstract Title:** Characterizing the metabolism of the deadly mushroom toxin  $\alpha$ -amanitin in mushroom-feeding *Drosophila*

**Authors:** Clare Scott Chialvo, Fariba Tayyari, Thomas Werner, Arthur Edison, Laura Reed,

**Presenting Author Affiliation:** University of Alabama

**Abstract Submission:**

The cyclopeptide toxin  $\alpha$ -amanitin, which is found in some *Amanita* mushrooms including the aptly named Death Cap and Destroying Angel, is responsible for more than 90% of all mushroom related fatalities. This toxin acts by binding to and inhibiting the function of RNA polymerase II (RNAP II) in the liver and kidney, causing irreversible damage and organ failure. While  $\alpha$ -amanitin is toxic to most eukaryotes, mushroom-feeding *Drosophila* can tolerate high doses of this compound and utilize toxic *Amanita* mushrooms as hosts. These species do not possess a mutation in RNAP II that would prevent  $\alpha$ -amanitin binding. In this study, we used global  $^1\text{H}$  NMR analyses to detect the metabolic fate of  $\alpha$ -amanitin in two tolerant *Drosophila* species (*Drosophila guttifer* and *D. recens*). We compared the metabolome of these species to one closely related susceptible species (*D. deflecta*). The tolerant species were reared on diets with and without  $\alpha$ -amanitin, while the susceptible species was only reared on a diet without the toxin. We compared the metabolomes of the three species using a principal component analysis. These analyses demonstrate that tolerant and susceptible species reared on diets without  $\alpha$ -amanitin exhibit distinct metabolic profiles. In addition, they identify the dietary concentration needed to detect the metabolic fate of  $\alpha$ -amanitin in tolerant species.

**Poster #: 349**

**Abstract #: 2113**

**Abstract Title:** Metabolic analyses of the calcification process in the marine microalgae *Emiliana huxleyi*

**Authors:** Robin Wördenweber, Jan Mussnug, Olaf Kruse,

**Presenting Author Affiliation:** Algae Biotechnology & Bioenergy Group, Center of B

**Abstract Submission:**

As a globally distributed, mineralizing organism, the calcifying marine microalga *Emiliana huxleyi* is of great interest for fundamental science and biotechnological applications. The cell is usually covered with coccoliths that consist of highly elaborate structures made of calcium carbonate and polysaccharides. Produced in a cell-free system, coccoliths could provide a new, high-quality composite material with potential applications in medical and material science.

In order to elucidate the process of coccolith formation, metabolic analyses were performed on a calcifying and on a non-calcifying cell state of *E. huxleyi*. Extensive metabolomics data sets were generated, containing intracellular primary metabolites, and more specifically lipid and pigment profiles, allowing detailed insights into the metabolism under nutrient replete and deficient conditions. The interpretation of this data in combination with transcriptomic data of the same strains (Rokitta et al., 2014) facilitates the understanding of intracellular processes in this marine microalgae and could lead to the identification of key components for calcification.

Recent results regarding the differences between the metabolome of the calcifying and non-calcifying cell states will be presented.

Literature: Rokitta S. D., Dassow P., Rost B., et al. (2014): *Emiliana huxleyi* endures N-limitation with an efficient metabolic budgeting and effective ATP synthesis. *BMC Genomics* 15 (1051), doi:10.1186/1471-2164-15-1051.

**Poster #: 350**

**Abstract #: 2177**

**Abstract Title:** Meta-data analysis of metabolite profiles reveals potential usability of cyanobacteria as models of plant photorespiration

**Authors:** Isabel Orf, Stefan Timm, Hermann Bauwe, Alisdair Fernie, Martin Hagemann, Joachim Kopka, Zoran Nikoloski,

**Presenting Author Affiliation:** MPI of Molecular Plant Physiology

**Abstract Submission:**

While photorespiration is conserved among cyanobacteria, algae, and land plants, it evolved to different levels of complexity in these organisms. The highest complexity is found in land plants, where the pathway involves several cellular compartments and respective transport processes. This complexity raises the question whether a simpler system, such as cyanobacteria, may serve as a model to facilitate our understanding of photorespiration. We conducted a meta-data analysis of publicly available metabolite profiles from the cyanobacterium *Synechocystis* sp. PCC 6803 and the land plant *Arabidopsis thaliana*. Different challenging facts had to be considered in the context of this meta-data analysis. As a unicellular prokaryote, *Synechocystis* is non-compartmented. This may simplify the study of photorespiration, but makes the comparison to the compartmented pathway of *Arabidopsis* complicated. In a eukaryotic cell, metabolites are often present in multiple compartments. State-of-the-art metabolite profiles, however, represent the overall status of combined sub-pools of eukaryote cells. Additionally, data sets comprised only partially overlapping sets of primary metabolites. Finally, technical artefacts had to be taken into account for normalization prior to data analysis as data sets were measured independently of one another. All of the above factors were considered for the adequate choice of normalization procedures and methods of meta-data analysis. We chose three methods of comparative data analysis: (1) We determined the mathematical distance between the samples from both organisms to identify corresponding behaviour. (2) We analysed the variability of metabolites within and between the species. (3) We investigated if metabolite ratios are comparable. Our results indicate that the metabolic signature of photorespiration is largely preserved between cyanobacteria and land plants. Hence, cyanobacteria can serve as prokaryotic models of plant photorespiration. Furthermore, the approaches of meta-data analysis for inter-species comparison of metabolite profiles used in this study are easily transferable to other scientific questions of comparative nature.

**Poster #: 351**

**Abstract #: 2259**

**Abstract Title:** Untargeted metabolomics elucidates the role of diet and triglyceride storage in *Drosophila melanogaster* larvae

**Authors:** Vishal Oza,

**Presenting Author Affiliation:** Student

**Abstract Submission:**

Untargeted Metabolomics has been used to identify altered metabolic pathways in disease state. Here we employ *Drosophila* as a model organism to evaluate various aspects of Metabolic Syndrome (MetS), a complex disease that increase the risk for heart disease and diabetes. The prevalence of MetS has been attributed to the westernized dietary habit and sedentary lifestyle. Our previous studies have established, diet as one of the important contributors to metabolic phenotypes. Using untargeted metabolomics we have isolated and identified global metabolites in *Drosophila* larvae. We then employed Random Forest algorithm to obtain important metabolites that differed between the High fat (HFD) and normal (ND) diet as well as between reaction norm phenotypes (flies that store more triglyceride on normal diet and flies that store less triglyceride on normal diet). We found that in flies fed on HFD had an upregulation of the omega fatty acid oxidation pathway which is an alternative to the more common beta fatty acid oxidation. Furthermore, there was no correlation observed between triglyceride storage phenotype and fatty acids, indicating diet played more important role over triglyceride phenotype. In conclusion, although untargeted metabolomics allows for elucidation of global metabolic profile, the lack of *Drosophila* specific metabolites and pathways database hinders the use of *Drosophila* as a model organism for metabolomics studies.

**Poster #: 352**

**Abstract #: 2362**

**Abstract Title:** Forced degradation and LC/MS profiling in search of bioactive metabolites in *Bacillus amyloliquefaciens* fermentates

**Authors:** Anders Honoré, Henrik Jensen, Niels Christensen, Panagiotis Chanos, Helle Jackson, Joseph Kozole, Jana Fischer,

**Presenting Author Affiliation:** DuPont Nutrition Biosciences ApS

**Abstract Submission:**

*Bacillus amyloliquefaciens* LSSAO1 (Baf) is a potent producer of secondary metabolites with biocontrol potential. The production includes classes of ribosomal and non-ribosomal molecules, e.g. lipopeptides and polyketides. Each of the classes contains structural diversity. As an example, lipopeptides vary in the peptide entity as well in the length and conformation of the acyl chain with the terminus being linear, iso or anteiso. Likewise, polyketides like difficidin appears as both difficidin and oxydifficidin and isomeric forms hereof.

Baf fermentates show antimicrobial activity against both Gram negative and Gram positive bacteria. The Fermentates are sensitive towards heat and pH treatment, where activity is almost completely lost after acidification (pH 4) and/or heat treatment. It remains relevant to learn which compounds are responsible for the anti-Gram-negative effect to optimize strain selection, fermentation and downstream processing for commercial production.

In search for bioactive compounds, a strategy was employed to expose one fermentate to systematic variations in pH, time and temperature to reduce anti-Gram-negative effect. The treated fermentates were subjected to measurement of bioactivity and chemical profiles by LC/MS. Based on measured chemical profiles and bioactivity, the strategy was to build uni-/multivariate model(s) for predicting the bioactivity from profiles. With predictive models, variable selection can serve to exclude compounds not contributing to the model, i.e. having a constant (or zero) influence on the changes observed. Hereby, the variable selection provided a short list of candidates, which then can be (crudely) isolated to establish casual relations by bioassay.

**Poster #: 353**

**Abstract #: 2372**

**Abstract Title:** Improved Global identifiability with orthogonal platforms of LC/Orbitrap-MS and GC/TOF-MS using IROA

**Authors:** Yunping Qiu, Michelle Reid, Robyn Moir, Ian Willis, Chris Beecher, Richard Yost, Timothy Garrett, Irwin Kurland,

**Presenting Author Affiliation:** Albert Einstein College of Medicine

**Abstract Submission:**

**Introduction:** We have shown that high mass accuracy GC/MS, in combination with Isotopic Ratio Outlier Analysis (IROA) was effective for both quantitation and discovery of novel metabolites for the *S. cerevisiae* metabolome (Anal Chem. 2016 Mar 188(5):2747-54). Here we have used GC/TOF-MS to synergistically expand LC/Orbitrap-MS coverage of the *S. cerevisiae* metabolome.

**Methods:** Yeast strains were grown to mid log phase on Yeast Nitrogen Base without amino acids, containing 5% or 95% <sup>13</sup>C glucose, and harvested at 20° C in 4:4:2 (v:v:v) methanol:acetonitrile:water, and analyzed using a Waters GC/TOF-MS, with a Fiehn library protocol. Extracts were also analyzed by LC/HRMS using a Thermo Q Exactive using an ACE Excel 2 C18-PFP column (100.2.1 mm, 2 μm), and fractions were pooled for 30 seconds during the run (total of 31 fractions), then dried for GC/MS derivatization. Mobile IROA peak pairs were identified for using Genedata Expressionist MS Refiner or Cluster Finder (IROA Technologies) software.

**Results:** Among the 58 metabolites of confirmed identity described using GC/TOF-MS, 42 were found in the LC fractions, 26 in fraction 1, 3 in fraction 2, 2 in fraction 3, 4 in fraction 4, and 1 in fraction 15.

Among 68 metabolites without confirmed identifications in our GC/TOF-MS study, 35 were found in the LC fractions, 24 in fraction 1, 6 in fraction 2, 3 in fraction 3, and 2 in fraction 4. The detection of IROA mirror pairs in the LC eluents for all the metabolites detected by GC/TOF-MS confirms biologic origins, without thermal degradation.

**Novel aspects:** These results confirm the usefulness of untargeted high mass accuracy GC/MS as an orthogonal partner to untargeted LC/MS, in contrast to a recent study by Siuzdak's group (Anal. Chem., 2015, 87:10935) which questioned whether GC/MS is picking up the desired compounds of a sample, or simply thermal degradation products.

**Poster #: 354**

**Abstract #: 2456**

**Abstract Title:** Multi-platform non-targeted deep metabolome annotation of the ecotoxicological and NIH model organism, *Daphnia magna*

**Authors:** Martin Jones, Tom Lawson, Ralf Weber, Clement Heude, Andrew Chetwynd, Warwick Dunn, Mark Viant,

**Presenting Author Affiliation:** University of Birmingham

**Abstract Submission:**

Mass spectrometry and NMR spectroscopy serve as workhorses to a thriving metabolomics research community. Innovations in separation and measurement technologies continue to advance our understanding of the diversity and complexity of low molecular weight metabolites within biological systems. The significant challenges of metabolite annotation and identification, however, often preclude the derivation of meaningful biological inferences from metabolomics datasets. Here we describe Deep Metabolome Annotation (DMA), a new approach designed specifically to address these challenges and, in turn, bridge the gap between high-throughput metabolomics data and derivation of meaningful biological knowledge. In collaboration with scientists from Thermo Fisher Scientific, we have devised and established an extensive workflow coupling physicochemical separations with NMR and MS<sub>n</sub> acquisitions to deeply annotate the metabolome of the model organism, *Daphnia magna* (water flea). This species serves as a sentinel organism in ecology and ecotoxicology, and is listed as one of only thirteen model organisms by the US National Institutes of Health. As input, the DMA workflow takes ten genetic strains of *Daphnia magna*, each cultured under two distinct environmental conditions (control and temperature/light stressed), which are all pooled into a single homogenate. Independent polar and apolar metabolite solvent extractions have then been undertaken and the resulting extracts fractionated over four SPE phases, yielding fifteen distinct SPE fractions. These fractions then undergo (a) multiple 1D and 2D NMR experiments (Bruker 600MHz), (b) further separation over four liquid chromatography columns (Thermo Scientific Ultimate3000) with concurrent time-based fraction collection, and (c) on-line tandem MS acquisitions (Q Exactive). Finally, the LC fractions are analysed by nESI-MS/MS<sub>n</sub> applying multiple collision modes and energies at high mass resolution (LTQ Orbitrap Elite). Results will be presented demonstrating the number and diversity of biochemical classes annotated using the DMA workflow, with comparisons to prior knowledge of the *Daphnia* metabolome derived from the literature.



**Poster #: 355**

**Abstract #: 2147**

**Abstract Title:** A murine sphingolipids atlas and its use in translational research

**Authors:** Federico Torta,

**Presenting Author Affiliation:** National University of Singapore

**Abstract Submission:**

Murine models are widely used to study mammalian biology. Lipids are crucial metabolites for healthy functioning of any organism. Sphingolipids are important components of the membranes (structural function) and also regulate various cellular activities (signaling function). Any disruption in the sphingolipid metabolism, enzymes or pathway can trigger pathological processes.

We are investigating (1) the sphingolipid composition of different murine tissues to create a reference atlas of the sphingolipidome in wild type C57BL/6 mice (2) the possible link between sphingolipid profiles and tissue functions (3) any gender-related difference in the murine sphingolipidome (4) the genetic basis for the differences in the sphingolipidome in different tissues (5) the perturbation of the sphingolipidome in pathological conditions that affect specific organs to find sphingolipid species that could be used as biomarkers or to clarify the mechanism of these pathologies (6) the reliability of our mouse atlas as a reference for studies on disease models. To define our atlas and to detect variations of the sphingolipidome in different conditions, a comprehensive list of sphingolipids (around 300 molecular species), including ceramides, sphingomyelins, glycosphingolipids, sphingoid bases and sphingosine phosphate(s) will be quantified by targeted LC-MS in all the samples examined.

**Poster #: 356**

**Abstract #: 2381**

**Abstract Title:** Automated kits and software for quantitative metabolomics

**Authors:** Danuta Chamot, Lu Deng, Rupasri Mandal, Trent Bjorndahl, Siamak Ravanbakhsh, Jason Grant, Michael Wilson, Beomsoo Han, Arnau Serra-Cayuela, Ying (Edison) Dong, Russell Greiner, David Wishart,

**Presenting Author Affiliation:** The Metabolomics Innovation Centre

**Abstract Submission:**

The automation of nuclear magnetic spectroscopy (NMR) and gas chromatography mass spectrometry (GC-MS) remains a pressing challenge in the field of metabolomics. Both are powerful techniques, but both are limited by the requirement of highly qualified personnel to operate equipment and to perform time-consuming manual spectral profiling. Our lab focusses on the design and development of easy-to-use NMR and GC-MS kits that will both streamline the operation and increase the throughput of metabolomics analyses. The goal of the kits is to provide everything, except the instrument, to run the analyses. The NMR kit contains all the necessary components to prepare an NMR sample for <sup>1</sup>H analysis, including a buffer solution and a deuterated internal standard. The NMR kit also contains detailed sample preparation instructions, as well as a user code for BAYESIL1, a web-based, automated, batch NMR spectral profiling web server. BAYESIL can automatically find the concentration of NMR-detectable metabolites accurately (~90% correct identification and ~10% quantification error), in less than 5 minutes. The NMR kit is currently designed to analyze serum, plasma and cerebrospinal fluid samples. The GC-MS kit consists of a derivatization reagent, internal and alkane standards (C8-C20 and C22-C40), as well as an instruction booklet to prepare and analyze samples for GC-MS analysis. The GC-MS kit contains a user code for GC-AutoFit2, an automated, web-based metabolite profiling tool. GC-AutoFit is designed to identify and quantify up to 127 compounds in urine, serum, plasma, CSF and milk. These metabolomics kits will enable a wealth of new applications of NMR and GC-MS in both laboratory and clinical settings.

**Poster #: 357**

**Abstract #: 2288**

**Abstract Title:** Multiplatform MS metabolomics reveals metabolic disorders in mice exposed to thirdhand tobacco smoke

**Authors:** Noelia Ramirez, Sara Samino, Sonia torres, Neema Adhami, Manuala Martins-Green, Xavier Correig,

**Presenting Author Affiliation:** Institut d'Investigacio Sanitaria Pere Virgili

**Abstract Submission:**

Thirdhand tobacco smoke (THS) is a novel and poorly understood pathway of tobacco exposure that is produced by the deposition and ageing of tobacco smoke particles and toxicants in surfaces and dust. This aged tobacco smoke becomes increasingly toxic with age, re-emitted into the air or react with other chemicals in the environment to yield new toxicants, including carcinogens. Furthermore, THS remains in indoor environments long after smokers move out, which makes THS a serious health problem, especially for children with smoking parents, which are the most vulnerable population to this pathway of tobacco exposure. Although the increasing evidences of THS hazards, the specific cellular and molecular consequences of exposure to THS remain to be fully elucidated. To address this, here we present the first non-targeted metabolomics approach applied to THS-exposed animal model: C57BL/6 mice, exposed to THS under conditions that mimic exposure of humans in homes of smokers. THS-exposed mice showed alterations in multiple organ systems including non-alcoholic fatty liver disease, inflammation in the lung, poor healing of cutaneous wounds, hyperactivity, hyperglycemia and insulin resistance in the form of non-obese type II diabetes (NODII) through oxidative stress. Serum and urine samples from THS-exposed mice and control ones were analyzed using GC-QTOF and reverse phase HPLC-QTOF. Accurate multivariate analysis revealed differently expressed metabolites. Interestingly, most of the altered metabolites coincide with those reported in metabolomics studies of current smokers and were related to oxidative stress, including glutathione cycle metabolites (glutamate and 5-oxo-proline). To map the metabolic changes, several mice tissues were analyzed using a novel matrix-free approach for Mass Spectrometry Imaging (MSI) based on sputtered gold nanolayers.

This study demonstrates the power of metabolomics for identifying the health hazards of THS exposure and, if confirmed in humans, would have a major impact on current tobacco health and environmental policies.

**Poster #: 358**

**Abstract #: 2034**

**Abstract Title:** Characterisation of the blood metabolome of dairy cows as affected by adaptation to lactation

**Authors:** Ákos Kenéz, Sven Dänicke, Martin von Bergen, Korinna Huber,

**Presenting Author Affiliation:** University of Hohenheim

**Abstract Submission:**

The metabolism of high-yielding dairy cows undergoes an enormous adaptation during the transition from late pregnancy to early lactation, driven by the requirements of milk production. According to their adaptation efficiency some cows develop severe metabolic diseases while others are able to maintain metabolic health. This study aimed to characterise changes of the blood metabolome during the transition period, and to identify healthy metabotypes within a cow herd.

Twenty-six German Holstein cows were used to collect blood samples repeatedly during the transition period: 42 and 10 days before calving and 3, 21 and 100 days after calving. Blood serum samples were subjected to a targeted liquid chromatography-mass spectrometry based metabolomics analysis using the AbsoluteIDQ p180 Kit of Biocrates Life Science AG (Innsbruck, Austria). Processed metabolomics data were evaluated by principal component analysis (PCA) in MetaboAnalyst and by heatmap visualisation in R.

The PCA revealed a strong separation of the data according to sampling days, indicating a notable shift of the metabolic pattern during the studied period. According to the heatmap, longitudinal adaptation of metabolism was mostly associated with changes of glycerophospholipids and sphingolipids, while clustering of cows was mostly determined by acylcarnitines. Amino acids and biogenic amines showed a more homogenous pattern with less variation over time.

Analysing longitudinal changes of the blood metabolome and identifying new biomarkers by this approach can help understanding the multifaceted metabolic adaptation of transition cows. The biological interpretation of the differences in blood acylcarnitine concentration may serve as a source for predicting healthy and diseased metabolic phenotypes in dairy cows.

**Poster #: 359**

**Abstract #: 2118**

**Abstract Title:** A metabolomics approach to study bacterial virulence in *Caenorhabditis elegans*

**Authors:** Michael Witting, Marianna Lucio, Romé Voulhoux, Steve Garvis, Reto Ossola, Andrea Amantonico, Cassandra Wigmore, Philippe Schmitt-Kopplin,

**Presenting Author Affiliation:** Helmholtz Zentrum München, Research Unit Analytica

**Abstract Submission:**

Metabolomics is the newest sibling in the “omics”-family. It aims to globally detect and (semi)quantify small molecules / metabolites present in a given system. We have setup a comprehensive metabolomics platform based on mass spectrometry (DI-FT-ICR/MS, UPLC-UHR-ToF-MS) enabling the simultaneous quantitative assessment of hundred known compounds within thousands of unknowns. MS data processing and analysis were automated using the Genedata Expressionist® software platform (Basel, Switzerland)

The nematode *Caenorhabditis elegans* is considered relevant for the study of bacterial pathogenesis as it is susceptible to a number of pathogens, which are able to kill or induce a range of symptoms of disease in the worm. The human pathogens *Pseudomonas aeruginosa* and *Salmonella enterica*, use virulence factors linked to human disease to infect and kill the nematode. Despite much research in this field, almost nothing is known regarding the metabolic response to infection in the worm.

We studied the *C. elegans* model with DI-ICR-FT/MS and UPLC-UHR-ToF-MS to investigate the unique metabolic phenotypes in worms facing specific stresses, infection or starvation. Our aim was to undertake whole worm quantitative metabolomics and recover specific markers of the induced metabolic changes brought on by interaction with pathogens. In this investigation, we reveal complex metabolic phenotypes allowing clustering based upon challenge.

Specifically we showed a significant decrease in amino acid metabolism during infection with *P. aeruginosa*, and a marked increase in sugar metabolism with infection by *S. enterica*. We were even able to discriminate between infection with a virulent wild type *P. aeruginosa* and an attenuated mutant. Results showed a direct effect on the worm’s energy and central carbon metabolism.

Our results show that the combination of non-targeted and targeted metabolomics is a promising tool for host-pathogen and *C. elegans* research ongoing studies with the worms are in the field of nutrition and ageing

**Poster #: 360**

**Abstract #: 2112**

**Abstract Title:**  $^{13}\text{CO}_2$  Isotope labelling for the elucidation of carbon fixation, metabolite transport and exudation processes in *Arabidopsis thaliana*

**Authors:** Karin Gorzalka, Hendrik Treutler, Gerd Balcke, Steffen Neumann, Dierk Scheel,

**Presenting Author Affiliation:** Leibniz-Institute of Plant Biochemistry

**Abstract Submission:**

Plants interact with their belowground environment by the secretion of compounds, a process called exudation. The exudation of metabolites is highly controlled and depends on numerous extrinsic (abiotic / biotic environment) as well as intrinsic factors, which influence the contents of e.g. phenolic compounds or their glycosylation patterns.

In this study, we used stable isotope labelling to track the metabolic processes for exudate generation from carbon fixation by photosynthesis over transport of compounds into the roots down to compound exudation including time dependent dynamics of this fixation and exudation process.

*Arabidopsis* plants were pulse labelled in a  $^{13}\text{CO}_2$  atmosphere for different labelling times. Shoots, roots and exudates were collected 1h, 3h, 6h, 9h, 20 and 23 hours after labelling and analyzed for primary and secondary metabolites using GC-MS and HPLC-MS, respectively. Metabolite labelling was quantified in the MS data by the comparison of isotope peak patterns from unlabeled control plants to labelled plants.

Besides manual targeted annotations, an untargeted analysis pipeline was developed for the automated detection and annotation of isotope patterns in GC-MS and LC-MS data. In LC-MS, this approach revealed up to 700 peak groups with about 1500 isotope clusters in shoots, roots and exudates. In GC-MS, up to 350 peak cluster with more than 1000 isotope patterns were detected. In addition, the GC-MS EI fragmentation provided the chance to cross-validate labelling patterns from several fragments of the same metabolite.

This dataset provides insight into the dynamics of a) carbon fixation in the aboveground plant, b) transportation of (labelled) metabolites into the roots and c) the exudation of metabolites. Time-dependent sampling furthermore resolves very interesting changes in metabolite labelling patterns after switches from light to dark phase and back to light.

**Poster #: 361**

**Abstract #: 2101**

**Abstract Title:** Software: Nexus Point of a Targeted Lipid Analyzer

**Authors:** JEAN-BAPTISTE VINCENDET,

**Presenting Author Affiliation:** SCIEX

**Abstract Submission:**

Software: Nexus Point of a Targeted Lipid Analyzer

Jean-Baptiste Vincendet<sup>1</sup>, Baljit K. Ubhi<sup>2</sup>, Sarada Tanikella<sup>3</sup> and Peter Zhu<sup>3</sup>

<sup>1</sup>SCIEX, Europe, <sup>2</sup>SCIEX, CA, USA and <sup>3</sup>Metabolon, North Carolina, USA

**Introduction:**

Software is the primary interaction for a user with a mass spectrometer for both data acquisition and results generation. The challenge of any sophisticated technology is the reduction of analytical complexity so as to maintain focus on the biological problem. The intricacy of quantitative lipidomics with isobaric interference and complicated sample preparation adds another layer for consideration. There is a need for a software-driven solution that frees the user to focus on the biological problem at hand rather than the generation and processing of data. Lipidomics Workflow Software was developed to manage sample receiving, sample preparation protocols, MS methods, and data processing of hundreds of MRM's for facile, quantitative lipid analysis. The software and its application are described herein.

**Methods:** Lipidomics Workflow Manager (LWM) Software is used to facilitate the targeted quantitation of 13 lipid classes. The software utilizes inputs from certificates of analysis from internal standard kits (Avanti Polar Lipids, STATE) to generate accurate preparation protocols for quantitative analysis. The software with validated acquisition methods was used to control the Lipidyzer<sup>TM</sup> Platform for targeted profiling. The data is automatically processed to yield 1. quantitative results for each lipid class 2. mole percent composition and 3. accurate lipid species compositions. As well, heat maps, QC charts and box and whisker plots are generated.

**Preliminary Data:**

Comprehensively covering these classes requires a two-injection approach but data for around 1200 lipid molecular species can be collected in around 20 minutes. Data was collected on a serum sample set to highlight reproducibility and robustness of the platform. Reports, statistical results and heatmaps are also shown – automatically processed after each batch of samples is collected.

**Poster #: 362**

**Abstract #: 2176**

**Abstract Title:** Global metabolomics reveals hydroxyacylcarnitine as a novel endogenous metabolic marker for CYP3A activity

**Authors:** Bora Kim, Sinae Kim, Jieon Lee, Andrew HyoungJin Kim, Joo-Youn Cho,

**Presenting Author Affiliation:** Department of Clinical Pharmacology and Therapeuti

**Abstract Submission:**

Cytochromes P450 (CYP) 3A, considered as the most important enzyme in drug metabolism, is often involved in drug-drug interactions (DDI). Endogenous metabolic markers of CYP3A activity are useful in clinical applications of CYP3A substrate drugs to predict DDI. For measuring CYP3A activity, midazolam has been considered as the probe drug and urinary 6 $\beta$ -hydroxycortisol/cortisol ratio and plasma 4 $\beta$ -hydroxycholesterol have been suggested as potential endogenous metabolic markers. This study aimed to identify new potential biomarkers which are correlated with midazolam clearance (CL) in both inhibition and induction states of CYP3A activity using global metabolomics.

Twenty-four men and twelve women received midazolam doses during three study phases: 1 mg midazolam alone (control phase) 1 mg midazolam after pretreatment with 400 mg ketoconazole once daily for 4 days (CYP3A-inhibition phase) and 2.5 mg midazolam after pretreatment with 600 mg rifampicin once daily for 10 days (CYP3A-induction phase). Plasma samples were collected 24h after midazolam administration and urine samples were collected at 12h intervals during the 24h before midazolam administration. Global untargeted analysis of plasma and urine samples was performed by ultra-performance liquid chromatography time-of-flight mass spectrometry and multivariate analysis.

Midazolam CL was highly correlated with a few medium-chain hydroxyacylcarnitines, 6 $\beta$ -hydroxycortisol, 16 $\alpha$ -hydroxytestosterone sulfate, and 16 $\alpha$ -hydroxyandrosterone sulfate (Pearson's correlation coefficient,  $r > 0.7$ ). From multiple regression analysis using these markers, an equation of prediction for midazolam CL was derived as follows:  $\ln(\text{CL}) = 2.2010 - 0.4453 * \text{GEND} + 0.06504 * 6\beta\text{-hydroxycortisol} + 0.03075 * \text{hydroxydecanoyl carnitine}$ , where gender = 1 if female and gender = 0 if male. An acceptable correlation between the predicted and observed midazolam CL was obtained with  $r^2 = 0.75$ . To confirm whether acylcarnitine is hydroxylated by CYP3A, we performed in vitro assay with human liver microsome and found that hydroxylation of fatty acids was decreased by treatment of ketoconazole, a CYP3A inhibitor. These results suggested that CYP3A could be



**Poster #: 363**

**Abstract #: 2213**

**Abstract Title:** Combined untargeted and targeted metabolomic profiling reveals urinary biomarkers for discriminating obese from normal weight adolescents

**Authors:** Kumsun Cho,

**Presenting Author Affiliation:** Seoul National University College of Medicine

**Abstract Submission:**

**Background:** Childhood and adolescent obesity may lead to obesity and related complications in adulthood. Biomarkers of obesity can be useful for screening for obesity complications and promoting early intervention during school age. Thus, the metabolomic differences in obese children and adolescents should be investigated for identification of potential biomarkers.

**Objectives:** We investigated urinary biomarkers to distinguish metabolomic characteristics between obesity and normal weight in adolescents.

**Methods:** Adolescent subjects were divided into nonobese (n = 91) and obese (n = 93) groups according to body mass index. Untargeted and targeted metabolomic profiling of urine was performed using high-performance liquid chromatography (LC)-quadrupole time-of-flight mass spectrometry (MS), and LC- and flow injection analysis-MS/MS systems, respectively.

**Results:** Multivariate statistical analysis showed clear discrimination between the untargeted metabolomes of nonobese and obese groups. Seven endogenous metabolites were distinguished in the obese group, and inflammation-related metabolite markers showed strong predictive power for group classification. From targeted metabolomics, 45 metabolites mostly related to inflammation were significantly different in the obese group.

**Conclusions:** Significantly different metabolome signatures were identified between normal weight and obese adolescents. Combined untargeted and targeted metabolomics demonstrated that inflammation-driven insulin resistance, ammonia toxicity, and oxidative stress may represent crucial metabolomic signatures in obese adolescents.

**Poster #: 364**

**Abstract #: 2275**

**Abstract Title:** Metabolic factors involved in Salmonella pathogenicity

**Authors:** Luca Rappez, Andrew Palmer, Ivan Protsyuk, Prasad Phapale, Bachir El Debs, Joel Selkrig, Nassos Typass, Theodore Alexandrov,

**Presenting Author Affiliation:** EMBL

**Abstract Submission:**

Salmonella enterica serovar Typhimurium is an intracellular pathogen which can infect enterocytes, intestinal epithelial cells specialized in nutrient uptake. Salmonella survives within enterocytes in specialized compartments, the Salmonella Containing Vacuoles (SCVs). Occasionally, Salmonella escape SCVs to the cytoplasm where they replicate at a much higher rate due to the higher nutrient abundance, described as the hyper-replication phenotype. While in the cytoplasm, Salmonella actively triggers host cell inflammation, leading to extrusion of the host cell from the epithelium to the intestinal lumen. The enterocyte then is lysed by pyroptosis, releasing inflammatory cytokines and free Salmonella which can infect other enterocytes. In addition, the entire metabolic content of the cell at the time of pyroptosis is exposed to the environment. The impact of these free metabolites on Salmonella pathogenicity has not been characterized yet.

This work is at an early stage and will study this process using metabolomics. We will present the early results with the aim to collect feedback from the community.

We will perform LC-MS/MS metabolomics in vitro by analyzing the extracellular medium. In particular, we will investigate how metabolites can impact the ability of Salmonella to re-infect new cells. This will be measured by using direct LC-MS/MS metabolomics and studying biological characteristics of the infection system. For data analysis, we will use our recently developed software Optimus (see the abstract by Ivan Protsyuk).

To obtain further information and make functional hypotheses on the roles of metabolites detected in LC-MS/MS, we are planning to perform MALDI-imaging mass spectrometry on infected epithelial monolayers. For metabolite annotation of MALDI-imaging data, we will use our bioinformatics recently developed in the framework of the European project METASPACE (see the abstract by Theodore Alexandrov). This will enable us to reveal the spatial distribution of these small metabolites, which can be mapped back to infected cells.

**Poster #: 365**

**Abstract #: 2299**

**Abstract Title:** Chemometric optimization of an LC-MS/MS method for the quantification of 70 selected metabolites related to mitochondrial health

**Authors:** Baldur Sigurdsson, Giuseppe Paglia, Sigurdur Smáráson,

**Presenting Author Affiliation:** Center for Biomedicine, European Academy of Bolzan

**Abstract Submission:**

Mitochondrial dysfunction has long been implicated in the etiopathogenesis of Parkinson's disease (PD), based on observations that mitochondrial toxins can cause parkinsonism. The first evidence came from specific cases of induced parkinsonism during the 1980s, when several drug users accidentally injected themselves with the synthetic heroine analog MPTP, an inhibitor of complex I in the mitochondrial respiratory chain. The diagnosis of PD is currently based on the clinical manifestations of the disease, and no established biomarker is available. Validated peripheral biomarkers would therefore be critically needed to achieve early diagnosis, therapy assessment, and classification of disease subtypes.

A quantitative metabolomics based assessment of the mitochondrial function could potentially lead to new diagnostic markers in PD. To this end 70 mitochondrial metabolites were identified from literature and metabolomics databases with the aim of developing a sensitive LC-MS/MS method for the quantification of all of them. Due to structural difference, the optimal conditions for each metabolite varies significantly. Therefore, a chemometric approach was used as a tool for finding the best compromise between optimal conditions. Two different experiments using MODDE 10.0 (Umetrics) DoE software were performed. First, a set of 34 screening experiments, based on D-optimal design were selected, to find the optimal LC column and solvent system. Second, a set of 83 experiments, based on face centered central composite design (CCF) with 8 variables, were selected for optimization. Experiments were correlated with LC-MS responses using partial least squares regression. The LC-MS system comprised of Shimadzu Nexera X2 coupled to a Sciex QTRAP 6500 triple quadrupole mass spectrometer. PeakView and MultiQuant (Sciex) were used to explore and process the datasets.

This method development demonstrates that utilizing DoE for optimization of LC-MS/MS methods is very efficient, with only fraction of the experiments required by changing one separate factor at time (COST) approach.

**Poster #: 366**

**Abstract #: 2437**

**Abstract Title:** Fingerprinting metabolomic approach by HPLC-ESI-QTOF-MS to find biomarkers of Mixed Connective Tissue Disease (MCTD): A preliminary study.

**Authors:** Isabel Borrás Linares, Álvaro Fernández Ochoa, Rosa Quirantes Piné, David Arráez Román, Marta Alarcón Riquelme, Ángel De la Torre Vega, Antonio Segura Carretero,

**Presenting Author Affiliation:** University of Granada

**Abstract Submission:**

Mixed Connective Tissue Disease (MCTD) is a kind of Systemic Autoimmune Diseases (SAD) which presents difficulties in its diagnosis due to the combination of symptoms and signs present in other SAD diseases such as scleroderma, polymyositis and systemic lupus erythematosus. Typical symptoms of this disease are raynaud phenomenon, kidney disease, joint inflammation, muscle weakness, and shortness of breath, among others.

The aim of this work was the search of possible biomarkers of MCTD in order to improve its diagnosis and treatment. For this purpose, a preliminary fingerprinting-based metabolomic study was carried out by analysing urine samples of MCTD and healthy controls patients.

Thus, urine samples were diluted with milli-Q H<sub>2</sub>O (1:10, v/v) and centrifuged before their fingerprinting analysis by HPLC-ESI-QTOF-MS. The metabolites were separated using a reversed-phase C18 analytical column (Agilent Zorbax Eclipse Plus, 3.5 µm, 2.1×150 mm) and detected in positive-ionization mode over a range from 100 to 1700 m/z.

Data were processed using Agilent MassHunter Qualitative software where molecular feature extraction (MFE) was performed. Data were filtered, aligned and normalized using percentile shift algorithm by means of Mass Profiler Professional software. Fold change analysis was applied to find metabolites which differ significantly between both groups of samples.

Results revealed a significant number of metabolites with differences between MCTD and healthy controls. These results represent a first attempt of finding possible biomarkers of MCTD which could help to design new experiments with a larger number of samples and in combination with other SAD in order to achieve improvements in diagnosis of this kind of diseases.

**Poster #: 367**

**Abstract #: 2467**

**Abstract Title:** Metabolomic biomarkers in saliva for the diagnosis of Alzheimer's disease

**Authors:** Ali Yilmaz, Stewart Graham, Ray Bahado-Singh,

**Presenting Author Affiliation:** Research Scientist

**Abstract Submission:**

Alzheimer's disease (AD) is a neurodegenerative disorder that is characterized by the accumulation of  $\beta$ -amyloid plaques and tau tangles. In contrast to AD, where other cognitive skills are affected, mild cognitive impairment (MCI) is defined by deficits in memory that do not significantly impact daily functioning. MCI is considered as a translational stage between normal aging and AD with MCI sufferers phenotypically converting to AD at a rate of 10% per year. Currently there is no cure and few reliable diagnostic biomarkers for AD. As we live longer there is an ever increasing demand for valid and reliable biomarkers of AD not only because it will help clinicians recognize the disease in its earliest symptomatic stages but will also be important for developing novel treatments of AD.

The potential of metabolomics to identify those at greatest risk of developing AD has been demonstrated previously (plasma and serum) however the novel use of saliva for AD diagnosis has not been previously reported. In this study we aim to demonstrate the potential of saliva as a potential non-invasive biofluid for the early detection of AD.

Combined with multivariate statistical analyses we employed both 1D and 2D NMR to biochemically profile saliva samples collected from healthy controls (n=10), MCI (n=10) and AD patients (n=10).

Using NMR we positively identified ~30 metabolites in saliva of which a significant number were found to be statistically significantly different ( $p < 0.05$ ). Using a combination of these metabolite concentrations we developed predictive models capable of distinguishing between control, MCI and AD patients.

**Conclusion:** This pilot study underscores the potential of using metabolomics and saliva for the early diagnosis of AD. We believe that the development of accurate biomarkers will assist in the detection of AD whilst promoting early intervention strategies when treatment is

**Poster #: 368**

**Abstract #: 2491**

**Abstract Title:** Distinct urinary metabolite profiles after strenuous exercise combined with casein intake

**Authors:** Lars Ridder, Lonneke Duijghuijsen, Richard Bas, Martie Verschuren, Jaap Keijer, Harry Wichers, Renger Witkamp, Klaske Van Norren,

**Presenting Author Affiliation:** Netherlands eScience Center

**Abstract Submission:**

Understanding the metabolic consequences of physical exercise is of crucial importance to improve prevention and treatment of age and disease related physical decline. Urinary metabolic breakdown products include potential biomarkers which have been poorly characterized so far. Here, we report on the application of urine metabolomics to study effects of strenuous exercise in combination with casein intake. The applied analytical setup and data analytics workflow allowed comprehensive characterization of the corresponding changes in the urine metabolome.

Twelve well-trained healthy male cyclists started the study protocol in fasted state. Urine was collected before and after ingestion of 40 grams of casein protein shake. After a strenuous exercise protocol[1], urine was collected, again before and after casein protein intake. This 2 day protocol was executed twice during two consecutive weeks. Each urine sample was analysed by full scan HILIC-MS, in positive and negative mode. Furthermore, pools of each sample group were analysed by HILIC-MS/MS. Peak detection and matching across LC-MS(/MS) profiles was performed to generate input for principal component analysis. MS/MS spectra were chemically annotated by the MAGMa software[2].

Principal component analysis (PCA) of all samples showed a clear distinction between urine metabolite profiles before and after intake of protein (PC1) and before and after exercise (PC2). Corresponding sample groups obtained in both executions of the protocol clustered together. Annotation of the MS/MS spectra was performed by automatic retrieval and ranking of candidate molecules from the human metabolite database (HMDB) using the MAGMa software. A number of identified compounds that strongly contributed to the separation of different sample groups are related to energy metabolism and muscle breakdown and their metabolic significance will be discussed in more detail.

[1] Carol et al. Int.J.Sport.Nutr.Exerc.Metab. 2011, 21, 135

[2] Ridder et al. Anal.Chem. 2014, 86, 4767

**Poster #: 369**

**Abstract #: 2533**

**Abstract Title:** IDENTIFICATION OF POTENTIAL BIOMARKERS IN PLASMA FROM PEDIATRICS WITH CHRONIC KIDNEY DISEASE BY MEANS OF MULTIVARIATE CHEMOMETRIC APPROACHES APPLIED TO LC-QTOF-MS BASED TARGETED METABOLOMICS DATA

**Authors:** alicia sanchez, Sandra Benito, nora unceta, ramon barrio, maria aranzazu goicolea, J.J. Jansen, G Postma, L.M.C. Buydens, fernando andrade, L Aldámiz-Echevarria,

**Presenting Author Affiliation:** University of the Basque Country (UPV/EHU)

**Abstract Submission:**

Chronic kidney disease (CKD) is considered to be a major worldwide public health problem. Nowadays, creatinine is the classic biomarker for the assessment of renal function in clinical practice. However, creatinine has several drawbacks, such as lacking sensitivity and revealing kidney damage when an important nephronic loss has already occurred. For that reason, there is a need for new biomarkers which could help for an earlier diagnosis, monitorization of the progression of the disease and evaluation of the response to therapy. Therefore, the goal of this study seeks to find specific biomarkers for pediatrics. [1,2].

Arginine-creatinine metabolic pathway, arginine methylation and urea cycle were suspicious to be affected in pediatric patients with CKD. Thus, a targeted metabolomics approach based on ion-pairing LC-QTOF-MS methodology was developed and optimized for the quantification of 16 metabolites, in order to find new potential biomarkers. This recently developed method was used to quantify the targeted analytes in plasma from thirty-two patients with different degrees of CKD and twenty-four control patients not suffering from CKD[3].

The results obtained have been analyzed following a multivariate approach, including scaling the data using different methods prior to Principal Component Analysis (PCA). It has to be noted that both including and not including creatinine concentration for building the models have been assayed. The effect of gender, age, different CKD stages and renal replacement therapy has also been examined. In addition, classification of the data with the aim of building predictive models for classifying future observations has also been carried out.

**References:**

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**Poster #: 370**

**Abstract #: 2549**

**Abstract Title:** Metabolic characterization of internet addiction disorder and discovery of integrative biomarker coupled to clinical parameters.

**Authors:** YEOUL CHO,

**Presenting Author Affiliation:** kookmin university

**Abstract Submission:**

Recently, the increased internet utilization leads to new type of mental and physical disability, internet addiction disorder. The disorder is generally defined as uncontrollable states which is pathologically addictive for video game, mobile device, and gambling. The abnormal status is clinically diagnosed by well-trained psychiatrists however, the authentic psychiatric diagnostic criteria does not exist since it has not yet categorized as “disease”. We analyzed blood plasma metabolites from the control group, IAD only, ADHD only, and IAD+ADHD using gas-chromatography time-of-flight mass spectrometry. Multivariate statistical model (OPLS-DA) with binary logistic regression suggested the single set of molecular indicators which can differentiate internet addiction from the control group and comorbid group (e.g. ADHD). Further we improved the model by integrating the endogenous molecular information with clinical parameters such as depression, anxiety, impulsivity, alcohol, attention.



**Poster #: 371**

**Abstract #: 2141**

**Abstract Title:** Biomarkers of Ageing in wild type and flavin monooxygenase 5 knockout mice

**Authors:** Dorsa Varshavi, Ian Phillips, Flora Scott, Elizabeth Shephard, Kirill Veselkov, Jeremy Everett,

**Presenting Author Affiliation:** Greenwich university

**Abstract Submission:**

Biomarkers of Ageing in wild type and flavin monooxygenase 5 knockout mice

Dorsa Varshavi<sup>1</sup>, Ian R. Phillips<sup>2</sup>, Flora Scott<sup>2</sup>, Elizabeth A. Shephard<sup>2</sup>, Kirill Veselkov<sup>3</sup> and Jeremy R. Everett<sup>1</sup>

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Ageing is a complex biological process which is associated with number of diseases, such as type-2 diabetes, cardiovascular disease and neurodegeneration [1]. The mechanism of ageing is not yet completely understood but ageing is characterized by a progressive decline in physiological and metabolic function [2].

We applied an NMR-based metabonomic approach to study the effects of ageing on the metabolic profiles of urine and plasma in wild type (WT) and in flavin monooxygenase 5 (FMO5) knockout (KO) C57BL/6 mice in which some characteristics of ageing were less pronounced [3]. A range of age-related biomarkers were identified in urine and plasma. Some metabolites showed similar patterns of changes with age, regardless of genetic background: others were altered only in KO or WT mice. Identification of these biomarkers of ageing could open up a new avenue of research to better understand the process of ageing and as such help extend the healthy human lifespan.

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**Poster #: 372**

**Abstract #: 2587**

**Abstract Title:** Metabomatching: A tool for untargeted identification of genetically determined metabolites

**Authors:** Roger Mallol,

**Presenting Author Affiliation:** University of Lausanne

**Abstract Submission:**

While still in its infancy, the use of metabolic profiles in genome-wide association studies (GWAS) has improved the detection of, and provided biological context to, the sometimes poorly understood effects of genetic variants on clinical phenotypes. While metabolite concentrations are generally the metabolome phenotype of choice, an untargeted approach, using binned NMR intensities as phenotype, presents a complementary alternative. Here, we present a metabolome- and genome-wide association study on <sup>1</sup>H-NMR urine profiles from 835 individuals of the Cohorte Lausannoise. We used an untargeted approach, with the NMR signal intensities as the study's phenotypes, thereby carrying all available metabolomic information into the study. We then identified the metabolites underlying significant association using, in addition to manual identification, a newly developed automated identification method (metabomatching). Metabomatching is based on the observation that a genetic variant often affects multiple features, and that this collection of features provides a signature (pseudo-spectrum) of the underlying associated metabolite. Comparing the pseudospectrum against experimental NMR spectra, listed in the Human Metabolome Database (HMDB), was an efficient method for identifying candidate metabolites. The two novel gene-metabolite associations resulting from this study are of particular interest because both involve metabolites which we also found to associate with clinical phenotypes. These associations thus provide specific, statistically-determined, evidence of the propagation of genetic effect from gene to disease via the metabolome.?

**Poster #: 373**

**Abstract #: 2302**

**Abstract Title:** A NOVEL LIPIDOMIC MODEL IN SERUM CORRELATES WITH THE BIOCHEMICAL AND MAGNETIC RESONANCE IMAGING RESULTS IN NAFLD DIAGNOSIS

**Authors:** Enara Arretxe, Raul Jimenez-Agüero, Marta Iruarizaga-Lejarreta, Luis Bujanda, David Balgoma, Maria J Perugorria, Emma Eizaguirre, Marcin Krawczyk, Frank Lammert, Jesus M Banales, Cristina Alonso,

**Presenting Author Affiliation:** One Way Liver

**Abstract Submission:**

Non-alcoholic fatty liver disease (NAFLD) is the most common cause of chronic liver disease worldwide. Until now, biopsy has been the gold-standard for NAFLD diagnosis, which is an invasive technique not exempt of complications. The development of non-invasive methods would improve the detection and monitoring of this disease, which is usually underdiagnosed due to the risks a biopsy carries for patients.

The aim of this study is to explore potential correlations between the biochemical measurement of hepatic triglyceride concentration (Folch value) and two different non-invasive methods for NAFLD diagnosis: Ultraperformance liquid chromatography coupled to mass spectrometry (UPLC-MS) of serum samples and Magnetic Resonance Imaging (MRI)-based method.

114 obese patients (BMI>35) were included in the present study. The grade of steatosis was estimated by histopathology and the biochemical measurement of the hepatic triglyceride concentration (mg of triglyceride/g of liver tissue) was performed according to the Folch method. The liver fat content was also determined by multi-echo MRI, and methanol and chloroform/methanol serum extracts were analyzed by UPLC-MS. A linear regression model based on the serum lipidomic profiling has been performed to generate a signature able to estimate MRI fat fraction and the Folch value. A forward stepwise method was selected as variable selection approach.

We found a lipidomic signature that correlates with MRI fat fraction and the hepatic triglyceride concentration (Folch value), and also shows a strong correlation with the grade of steatosis in these obese patients.

**Poster #: 374**

**Abstract #: 2059**

**Abstract Title:** LC-MS based untargeted metabolomics approach to explore biomarkers of aging in rat serum

**Authors:** ERHAN SIMSEK, Sam Fong Yau Li,

**Presenting Author Affiliation:** Chemistry Dept, NUS

**Abstract Submission:**

Aging is a complicated phenomenon involving gradual accumulation of various 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. Application of metabolomics technologies to the study of aging is a relatively new area and may lead to biomarkers for the aging process. To explore the metabolic changes involved in aging process, Sprague Dawley (SD) rats were chosen as model organism. Serum samples from healthy SD rats of 3, 6, 9 and 12 weeks were analyzed. After deproteinization, the samples were analyzed using liquid chromatography coupled with quadrupole time-of-flight mass spectrometry in ESI positive and ESI negative mode. Pooled quality control samples were also analyzed 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 principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (O-PLS-DA) revealed separation among different age groups involved in the study. Metabolic features responsible for the separation between groups were chosen based on their variable importance for the projection (VIP) values and univariate statistics. They were searched against accurate mass metabolite libraries of METLIN and Human Metabolome Database (HMDB) for identification. Pathway analysis on the identified features revealed a number of metabolites that were involved in the lipid metabolism. The study provides new insights into the mechanism and biomarkers of aging process.

**Poster #: 375**

**Abstract #: 2075**

**Abstract Title:** Sphingolipid Profiling using Robust and Sensitive LC-MS-MS Method

**Authors:** Shachi Saluja, Dietrich Merkel, Ujjaini Dasgupta, Dipankar Malakar, Manoj Pillai, Avinash Baja,

**Presenting Author Affiliation:** RCB, Faridabad, India

**Abstract Submission:**

**Introduction:**

Sphingolipids are a class of diverse biologically active compounds having a sphingoid backbone. They are the major constituents of the lipid rafts in the cell membrane and contribute to its structure and fluidity. Its regulated functions have specific roles in cancer initiation, progression and therapy. Owing to this sphingolipid profiling may be indicative of various types of cancer.

**Methods:**

Lipids from variety of clinical samples were extracted following the published protocol and subjected to LC/MS analysis using LC 800 system from GL Sciences coupled with 4500 QTRAP® mass spectrometer (SCIEX). MRM methods for all 54 target compounds were developed. Two different mass spectrometry workflows were applied during data acquisition: MRM triggered Enhanced Product Ion (EPI) mode as well as Precursor Ion (PI) scan triggered EPI mode. LipidView™ software was used for lipid identification, MultiQuant™ software for lipid quantification.

**Preliminary Data:**

A total of 29 different classes of sphingolipids in human breast cancer cell line (MCF-7) were identified and quantified using the MRM triggered EPI workflow. Among these lipid species, sphingosine, which is known to be low abundant in nature, was detected with high confidence. In addition, a total of 44 different sphingolipid species obtained from Colon Cancer Cell line (HCT8) was identified and quantified. Low abundant N-acyl ceramides, Di-hydro ceramides, Glucosyl ceramides, ceramide 1 phosphate etc. could be identified using such workflow. After calculating peak areas for each of the lipid species in different technical replicates a good reproducibility could be shown with CV values below 15%. To understand the limit of detection a non-naturally occurring internal standard (sphingophosphorylcholine) was spiked into the sample and analysed using the same method. A LOQ of 80 pg on column was achieved. In addition, two Ganglioside species were identified by Precursor Ion (264.1) / EPI scanning experiments.

**Poster #: 376**

**Abstract #: 2125**

**Abstract Title:** NMR based metabolomics of exhaled breath condensate to differentiate between asthma, chronic obstructive pulmonary disease and asthma-chronic obstructive pulmonary disease overlap syndrome

**Authors:** Nilanjana Ghosh, Elavarasan Subramani, Mamta Joshi, Saumya Samanta, Parthasarathi Bhattacharyya, Rintu Banerjee, Koel Chaudhury,

**Presenting Author Affiliation:** Indian Institute of Technology, Kharagpur

**Abstract Submission:**

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. ACOS presently remains a diagnostic challenge for clinicians since there exist no specific biomarkers to differentiate ACOS from asthma or COPD. Metabolomics of exhaled breath condensate (EBC) has emerged as a popular non-invasive tool for the identification of metabolic fingerprints that can effectively discriminate between various clinical conditions. We hypothesize that NMR metabolomic analysis of EBC will help classify ACOS patients better and ascertain whether this overlap syndrome has its own clinical entity. EBC samples were collected from patients with asthma (n=33), COPD (n=30) and ACOS (n=38), classified as per Global guidelines. Proton NMR spectra of EBC samples were acquired using 800 MHz Bruker Avance III spectrometer equipped with a CryoProbe. Following phase and baseline correction, multivariate analysis was applied and individual metabolites identified. This study reports for the first time EBC metabolomics of patients with ACOS. On comparing asthma, COPD and ACOS, an optimum classification of ACOS was obtained with the OPLS-DA model. Values of R<sup>2</sup>Y and Q<sup>2</sup> validate that the OPLS-DA model fits well with the training data set and is able to predict the classes better than chance. Further, validation with permutation test statistics indicates that the original model has better predictive capability than the 100 generated permuted models. Finally, receiver operating characteristic curve could identify with highest accuracy the metabolites responsible for distinguishing ACOS. Metabolites including acetate, ethanol, fatty acid, glutamate, glutamine, lactate, leucine, pyruvate, succinate, proline, trimethylamine, valine, isobutyrate, acetone, 2-propanol, propionate, acetoin and methionine were significantly dysregulated in ACOS patients. These metabolites are largely associated with glycolysis pathway intermediates, amino acid biosynthesis and lipid/fatty acid metabolism.

**Poster #: 377**

**Abstract #: 2350**

**Abstract Title:** Increased fructose consumption and inadequate copper intake on the pathogenesis of nonalcoholic fatty liver disease (NAFLD): A feces and liver metabolomics study

**Authors:** David Alonso, Xiaoli Wei, Ming Song, Xinmin Yin, Biyun Shi, Joe Binkley, Michelle Page, Craig McClain, Xiang Zhang,

**Presenting Author Affiliation:** LECO Corporation

**Abstract Submission:**

Increased fructose consumption and inadequate copper intake are two critical risk factors in the development of nonalcoholic fatty liver disease (NAFLD). To investigate whether copper deficiency plays a role in fructose-induced fatty liver, male weanling Sprague-Dawley rats (35–45 g) were housed in stainless steel cages in a temperature and humidity controlled room with a 12:12 h light:dark cycle. All rats were allocated randomly into four groups. Each group of rats was fed either an adequate copper or marginally copper deficient diet. At the same time, distilled water or distilled water containing 30% fructose (w/v) was given ad lib for 4 weeks. Fructose enriched drinking water was changed twice a week. At the end of the experiment, all the animals were euthanized under anesthesia with pentobarbital (50 mg/kg I.P. injection). Liver and feces were collected and snap-frozen with liquid nitrogen for metabolomics study.

Metabolites were extracted using a solvent mixture of methanol and water. These compounds were derivatized using N-(tert-butyldimethylsilyl)-N-methyltrifluoroacetamide (MTBSTFA). Samples were analyzed using comprehensive two-dimensional gas chromatography (GCxGC) with time-of-flight mass spectrometry. Experimental data were processed with software for peak determination, deconvolution and compound identification, followed by MetPP software for retention index matching and metabolite quantification. To further confirm the identification of metabolites with significant abundance changes between sample groups, two samples from each group were selected and their metabolite extracts analyzed using GCxGC coupled to a high resolution time of flight mass spectrometer. Data was processed and interrogated retrospectively for key metabolites using Target Analyte Finding methodology. High resolution, accurate mass data for molecular, adduct, and fragment ions were leveraged for rapid data processing and confident metabolite characterization. Preliminary analysis of rat liver and feces samples demonstrated a significant metabolite profile difference between sample groups.

**Poster #: 378****Abstract #: 2377**

**Abstract Title:** Urine targeted LC-MS/MS profiling of hydrophilic metabolites in neonates with late onset sepsis

**Authors:** Olga Begou, Anastasia Chrysovalantou Chatziioannou, Kosmas Sarafidis, Agathi Thomaidou, Helen Gika, Aggeliki Kontou, Elisavet Diamanti, Charalambos Agakidis, Vasiliki Drossou, Nikolaos Raikos, Georgios Theodoridis,

**Presenting Author Affiliation:** Department of Chemistry, Aristotle University of T

**Abstract Submission:**

Despite advances in neonatal care over the last decades, neonatal sepsis remains a very serious threat for the health and life of neonates. The main difficulty in diagnosing microbial late-onset (LOS) sepsis is the fact that symptoms are non-specific similar to those of non-infectious conditions. Most of the known biomarkers evaluated in LOS lack high diagnostic accuracy hindering early and prompt initiation of treatment. Early and accurate diagnosis of sepsis is crucial for its management. The aim of our project was to determine significant changes of the metabolites in septic neonates as compared to non-septic ones (controls) hospitalized in the NICU setting using liquid chromatography-tandem mass spectrometry (LC-MS/MS). Development of such profile could possibly enable fast analysis with robust and reliable results assisting thus clinicians in decision making.

Urine samples were collected at the time of initial diagnosis of LOS from 18 neonates with LOS and 18 control neonates. All metabolites were analysed by UHPLC-MS/MS involving extraction of 50  $\mu$ L urine with acetonitrile (deproteinization). Chromatographic separation was performed on a Acquity BEH Amide column (150 $\times$ 2.1mm i.d., 1.7  $\mu$ m) with a flow rate of 0.5 mL/min. Mass spectrometry parameters were optimized for each of the 108 pre-selected analytes (amino-acids, organic acids, sugars, nucleosides, amines and others). Data were processed with multivariate (SIMCA 13) and univariate statistics (ANOVA).

PLS-DA showed clear separation between septic and control neonates. Statistical models were tested for validity with permutation and other tests. Visualisation plots (S-plots, VIP, box plots) and ANOVA verified noteworthy variations in the levels of 24 metabolites.

Results of the metabolomics analysis showed that neonates with LOS show different metabolic profiles from those without sepsis allowing their discrimination with the use of LC-MS/MS-based urine metabolomic analysis. Monitoring and quantitation of specific metabolites could help clinicians in applying the optimal treatment in septic neonates.



**Poster #: 379**

**Abstract #: 2378**

**Abstract Title:** ROCK inhibition affects the metabolic physiology of human pluripotent stem cells

**Authors:** Spyros Vernardis, Athanasios Mantalaris,

**Presenting Author Affiliation:** Biological Systems Engineering Laboratory, Departm

**Abstract Submission:**

Human pluripotent stem cells (hPSCs), embryonic (hESCs) and induced pluripotency (hiPSCs), offer enormous potential as suitable cell source in cell-based therapies due to their unlimited self-renewal and differentiation to all cell types properties. To harness the immense potential of hPSCs requires highly controlled and reproducible culture conditions (Placzek et al, 2009). For instance, hPSCs, being adherent cells, require the formation of colonies in order to maintain their self-renewal and stemness. Standardization of differentiation protocols necessitates single cell cultures, which has been recently achieved through colony dissociation and addition of ROCK (Rho kinase) inhibitors (Zhang et al, 2011 Watanabe et al, 2007). Although it has been shown that hPSCs retain their stemness, little is known about the dynamic changes in their physiology following exposure to ROCK inhibitors. Herein, we evaluated the effect of 10 $\mu$ M ROCK inhibitor (Y-27632) addition in both hESCs and hiPSCs cultures for variable exposure times: 0h-untreated, 12h, 24h, 48h and 96h. Whereas pluripotency marker, TRA-1-81+/SSEA3+ (flow cytometry) and Nanog, Oct4 and Sox2 (qRT-PCR), expression remained constant throughout (96h), metabolite profiles revealed differences in cellular metabolic physiology immediately following treatment (12h) with Y-27632 in both hESC and hiPSC cultures. Specifically, changes in glycolysis, TCA cycle and amino acids pools were observed as a result of treatment, revealing the need of the cells to adapt in the new culture conditions. These results demonstrate that dynamic metabolomics analysis provides the sensitivity required for the accurate characterization of cellular physiology and can contribute to the optimization of stem cell bioprocesses.

**Poster #: 380****Abstract #: 2409**

**Abstract Title:** "PARADISE" a novel allinone software for automated chromatographic peak deconvolution, identification, and quantification

**Authors:** Lea Johnsen, Morten Danielsen,

**Presenting Author Affiliation:** MS-Omics

**Abstract Submission:**

Reliable and high throughput peak detection and quantification is one of the most important and challenging aspects of metabolomics studies involving hyphenated analytical platforms such as GCMS. PARAllel Factor Analysis<sup>2</sup> based Deconvolution and Identification System (PARADISE) is particularly developed for modelling threeway datasets such as GCMS for extracting maximum metabolite information. PARADISE is based on the well-established multiway data modelling technique PARAllel FACtor analysis 2 (PARAFAC2) and combines it with semi-automated metabolite identification. It allows users rapid inspection and processing of complex GCMS datasets by utilizing advanced chemometric methods. A major advantage of utilizing threeway modelling is that all samples are included in one model. Thus, validation of every sample is not required. When analysing many samples this increases productivity dramatically.

PARADISE has been used to analyse a wide variety of GCMS data sets. Some of these datasets had already been analysed with traditional software. In all of these cases, new compounds were found, and obtained spectra was, in general, more pure and gave better matches when library searches was performed.

In summary, we find that by using PARADISE for analysing GCMS data the following advantages are obtained:

- no need for prior data pre-treatment (raw data can be used)
- peak deconvolutions are performed without user defined parameters
- high throughput processing of hundreds of samples simultaneously
- retention time shifts of peaks are allowed
- low signal to noise peaks can be resolved
- baselines can be modelled and excluded
- co-eluting and overlapping peaks can be separated and pure mass spectra are obtained In this way all components can be disentangled completely which provide interference free mass spectra and improve identification and quantification of true relative concentrations of deconvoluted peaks - All of the above is performed in a user friendly graphical interface

**Poster #: 381**

**Abstract #: 2413**

**Abstract Title:** GC-MS based metabolomics of Fusarium head blight using near-isogenic lines of wheat

**Authors:** Alexandra Parich, Benedikt Warth, Christoph Bueschl, Denise Schoefbeck, Bernhard Kluger, Rudolf Krska, Marc Lemmens, Gerhard Adam, Rainer Schuhmacher,

**Presenting Author Affiliation:** BOKU University Vienna, IFA-Tulln

**Abstract Submission:**

Fusarium graminearum commonly infests grains resulting in the devastating plant disease Fusarium head blight (FHB) and the formation of mycotoxins. The disease is difficult to control and resistance to FHB is mediated by numerous host genes.

In this study we used a GC-MS-based targeted metabolomics approach to investigate the metabolic response of wheat upon inoculation with the pathogen *F. graminearum*. For this purpose, near isogenic wheat lines (NILs) (98% identical genome) differing in the presence/ absence of the two known qualitative trait loci (QTLs) Fhb1 and Qfhs.ifa-5A, which partly confer resistance against FHB, were challenged at anthesis in two inoculation variants: water (control) and spores of *F. graminearum*. For each NIL and treatment, five wheat ears were harvested at 0, 12, 24, 48 and 96h after inoculation. The samples were immediately frozen in liquid nitrogen and stored at -80°C until analysis. Frozen wheat ears were homogenised in a ball mill (lq. nitrogen), extracted and analysed by a two-step derivatization GC-MS protocol. Data evaluation was carried out with the latest version of the freely available MetaboliteDetector software.

Unsupervised multivariate statistics revealed a clear separation of *Fusarium graminearum* treated and water treated samples. In addition to shikimic acid the aromatic amino acids tryptophan and phenylalanine which form precursors of major secondary defence metabolites were induced by *F. graminearum* and showed steadily increasing levels over time. Moreover the amino acid ornithine, the sugar phosphate glucose-6-phosphate, as well as the biogenic amines putrescine, tryptamine and serotonin which are both stress markers were induced under infection conditions.

This contribution will not only relate differentially formed substances to metabolic pathways but also link these metabolites to defined genetic resistance markers (QTLs) in the wheat genome.

**Poster #: 382**

**Abstract #: 2422**

**Abstract Title:** Statistical Criteria for Testing Metabolites and Prediction Rules on Stability over Time in One-Time Urinary Metabolomic Measurements

**Authors:** Johannes Hertel, Nele Friedrich, Maik Pietzner, Katharina Wittfeld, Kathrin Budde, Sandra Van der Auwera, Alexander Teumer, Lara Strobel, Robin Winkelmann, Hans Liebscher, Thomas Kocher, Matthias Nauck, Hans Grabe,

**Presenting Author Affiliation:** Department of Psychiatry and Psychotherapy, Univer

**Abstract Submission:**

The human metabolome is a dynamic system and modeling these dynamics could enhance stability and interpretability of metabolomic results. However, methods evaluating these dynamics in onetime metabolomic measurements are missing. Here, we propose a model for metabolite concentrations in blood and urine, utilizing a time dependent differential equation that models the kidney processes glomerular filtration, tubular reabsorption and secretion. The analytical solution of the differential equation was then transformed to a statistical model, applicable to one-time urinary data. We showed mathematically that for urinary metabolites under the steady state model stochastic independence on glomerular filtration and the normalization variable after correcting for urinary dilution must be given, delivering testable statistical criteria. In the case of departure from the physiological steady state, the model allowed specific predictions on the statistical associations of a metabolite with these variables. As metabolomic models are usually linear combinations of metabolite concentrations the criteria can be used to test the prediction rules as well as the metabolites on stability over time without sampling longitudinal data. The consistency of the model was tested via its explicit predictions on a large probabilistic quotient normalized urinary <sup>1</sup>H-NMR metabolomic data-set from the general population (Study of Health in Pomerania, n=4068). From 59 quantified metabolites 53 showed model consistent results with 49 being systematically influenced by glomerular filtration or the normalization process to account for dilution of urinary samples, both in a way predicted by our model and by corresponding simulated data. Four metabolites were consistent with the steady state model. Extensions of our framework could have many applications in metabolomics by providing testable hypotheses on parameters influencing the metabolome, complementing the hypothesis-free data mining procedures by a theory driven approach. In conclusion, differential equation based modeling might prove to be valuable tool even in one-time metabolomic measurement.

**Poster #: 383**

**Abstract #: 2429**

**Abstract Title:** Targeted metabolomics to identify links between ageing and musculoskeletal health in humans – A pilot study

**Authors:** Daniel Wilkinson, Matthew Swift, Philip Atherton, Ken Smith,

**Presenting Author Affiliation:** University of Nottingham

**Abstract Submission:**

The development of mass spectrometric approaches, in particular metabolomics, to look for prognostic biomarkers of health and disease is progressing rapidly, with certain metabolite profiles showing promise as diagnostic biomarkers in a number of chronic disease states (CVD, diabetes). With an increasingly ageing population, and the known relationships between ageing muscle, metabolic dysfunction and chronic disease states, metabolite biomarkers could help identify those individuals at greater risk of developing disease as they age. The aim of this study was to run a small GC-MS targeted metabolomics analysis to study the relationship between plasma amino acid profiles from young (N=17, age: 18 – 28y) and old (N=21, age: 66 – 73y) healthy male volunteers, and indices of their muscle mass and function. Fasting plasma samples were selected at random for full amino acid profiling using GC-MS following derivatization as their tBDMS esters. Using correlative analysis both appendicular skeletal muscle mass (ASM) and skeletal muscle index (SMI) displayed significant correlations between their respective indices and the BCAAs ( $r=0.412$ ,  $p=0.01$  and  $r = 0.439$ ,  $p=0.006$  respectively), with body mass index (BMI) showing a trend towards significant correlation ( $r = 0.264$ ,  $p=0.061$ ), indicating a relationship between higher levels of muscle mass and plasma levels of BCAAs. Further analysis revealed that these correlations were primarily driven through the younger volunteers (BMI young:  $r=0.695$ ,  $p=0.002$ , SMI young:  $r=0.517$   $p=0.033$ ). Similar findings were observed with strength measures, with BCAAs having a significant positive correlation with 1-RM, which when normalised for age showed a trend to significance in young ( $p=0.091$ ). These initial pilot findings highlight a promising link between plasma levels of BCAAs, age and indices of muscle mass and function. Thereby suggesting an important potential role for metabolomics in identifying novel biomarkers in order to develop a better understanding of the patho/physiology of the musculoskeletal system across the lifespan.

**Poster #: 384**

**Abstract #: 2430**

**Abstract Title:** Seeking Biomarkers for Niemann-Pick Type C1 Disease: A <sup>1</sup>H NMR-Linked Metabolomics Study

**Authors:** Martin Grootveld, Victor Ruiz-Rodado, Danielle te Vrugte, Daniel Sillence, Fay Probert, David Elizondo, Robin Lachmann, Frances Platt,

**Presenting Author Affiliation:** De Montfort University

**Abstract Submission:**

**Introduction/Objectives:** Niemann-Pick type C (NP-C) disease is an autosomal recessive, neurodegenerative lysosomal storage disorder which presents with a range of clinical phenotypes. Currently, biofluid biomarkers available for NP-C disease and its prognosis are limited. Therefore, in this investigation we have employed a global high-resolution <sup>1</sup>H NMR analysis strategy coupled to a range of exploratory data analysis and pattern recognition techniques in order to seek and identify such biomarkers in urine.

**Methods:** Urine samples were collected from a group of untreated NP-C1 disease patients, and also from corresponding heterozygous (parental) carrier control participants (n = 55 in total). <sup>1</sup>H NMR spectral profiles of these samples were acquired on a Bruker AV-600 spectrometer (Queen Mary University of London facility). Multivariate metabolomics analysis of the datasets acquired featured a range of approaches [specifically PCA, PLS-DA, Random Forest and Logistic Correlated Component Regression, together with Genetic Algorithm (GA) techniques].

**Results:** <sup>1</sup>H NMR-linked metabolomics analysis revealed (1) significantly elevated creatinine-normalised urinary excretions of bile acids, branched-chain amino acids (BCAAs), the BCAA and thymine catabolite 3-aminoisobutyrate (3-AIB), glutamine, 3-methylhistidine, quinolinate and trimethylamine (2) diminished concentrations of nicotinate, N-methylnicotinamide and trigonelline and (3) the presence of abnormal N-acetylsugar/saccharide fragments in NP-C1 patients. The multivariate analysis strategies employed achieved high levels of disease classification success rates (90-98%). These results provided evidence for NPC1 disease-mediated imbalances in pathways involved in bile acid biosynthesis, BCAA degradation and thymine catabolism, in addition to those featured in the nicotinate and nicotinamide, methylamine and amino-sugar metabolic pathways. Metabolite set enrichment and pathway topological analyses indicated that the lysosome, brain and liver represented key organ, tissue and sub-cellular localisations for this disease process, observations consistent with NP-C1 disease pathology.

**Conclusions:** Urinary 3-AIB and selected bile acids, N-acetylsugars/saccharides and/or BCAAs may serve as valuable biomarkers for the diagnosis and prognostic/therapeutic monitoring of NP-C1 disease patients.

**Poster #: 385**

**Abstract #: 2516**

**Abstract Title:** GC/MS and <sup>1</sup>H-NMR metabolomic approach for the study of IBD patients

**Authors:** Maria Laura Santoru, Cristina Piras, Antonio Murgia, Sonia Liggi, Pierluigi Caboni, Paolo Usai, Aldo Manzin, Tonina Lai, Luigi Atzori,

**Presenting Author Affiliation:** University of Cagliari

**Abstract Submission:**

Inflammatory bowel disease (IBD) represent a chronic disorder affecting one or more parts of the intestine, whose idiopathic aetiology is still not entirely known. The two major phenotypes of IBD are Crohn's disease (CD) and ulcerative colitis (UC). There is no specific diagnostic test for IBD and diagnosis is mainly based on integration of endoscopic, radiologic, histologic and laboratory data with the evaluation of the medical case and the degree of disease activity. A metabolomics approach was applied to study faeces and plasma of patients affected by IBD and healthy controls, to investigate the metabolic pathways involved in IBD and the role of metabolites as potential biomarkers.

A total of 183 samples of faeces and plasma were collected, out of which 50 from patients with diagnosis of CD, 82 from patients suffering from UC, and 51 from healthy controls. Faecal samples were analysed both by Nuclear magnetic resonance (<sup>1</sup>H-NMR) and Gas chromatography mass spectrometry (GC/MS), while plasma samples were studied by GC/MS only. Data analysis was carried out using orthogonal partial least squares-discriminate analysis (OPLS-DA) by using SIMCA.

The OPLS-DA models showed clear separation of CD and UC from healthy controls for both plasma and faeces samples exhibiting significant differences in the metabolic profiles and passed the permutation test. The metabolites holding differential power primarily belonged to a range of amino acids, sugars and biogenic amines. On the other hand, the comparison between UC and CD did not show a good separation indicating a similar metabolic profile in the two major IBD conditions.

In conclusion, this study underlines the potential role of metabolomics in the understanding of the metabolic pathways involved in IBD and its pathogenesis.

**Poster #: 386**

**Abstract #: 2557**

**Abstract Title:** Identification of plasma metabolic indicators of sepsis and sepsis severity using high-resolution accurate-mass and fragmentation profiles

**Authors:** Julian Avila, Amanda Souza, Shuba Gopal, Sarah Jeanfavre, Ralf Tautenhahn, Tim Stratton, Nathan Shappiro, Clary Clish,

**Presenting Author Affiliation:** Broad Institute

**Abstract Submission:**

Sepsis is characterized by an overwhelming and often life-threatening response to infection. Both specific diagnostic indicators of incident sepsis and prognostic indicators of progression to severe sepsis and septic shock are needed in the clinic. Here, we applied high-resolution accurate-mass metabolomic profiling to baseline plasma samples collected from 136 emergency department patients, including 82 subjects with varying degrees of sepsis and 54 non-infected controls, to identify novel metabolic indicators of incident sepsis as well as predictors of sepsis severity. Hydrophilic interaction liquid chromatography (HILIC) was used to separate polar metabolites and profiles for experimental samples and quality control pooled plasma samples were acquired using high resolution full MS scanning in the positive ion mode. This method measured over 90 plasma metabolites of known identity that had been previously confirmed using authentic reference standards as well as thousands of LC-MS peaks from yet to be characterized metabolites. To address the challenge of identifying significantly dysregulated unknowns, MS/MS spectra of isolated precursor or product ions in pooled QC samples were acquired using data dependent MS<sup>n</sup> scanning. Compound Discoverer™ 2.0 was used to conduct differential analysis among groups and to facilitate identification of unknowns using both precursor ion accurate mass searching against metabolite databases and batch searching of fragmentation spectra against a cloud-based MS spectral database. These analyses revealed several modified nucleosides, believed to be derived from tRNA, that predict both development of sepsis and progression to septic shock.



**Poster #: 387**

**Abstract #: 2051**

**Abstract Title:** Complementary fingerprinting of a human thyrotoxicosis model using proteomics and metabolomics

**Authors:** Maik Pietzner, Beatrice Engelmann, Tim Kacprowski, Janine Golchert, Anna-Luise Dirk, Georg Homuth, Elke Hammer, Matthias Nauck, Henri Wallaschofski, Thomas Münte, Nele Friedrich, Uwe Völker, Georg Brabant,

**Presenting Author Affiliation:** Institute of Clinical Chemistry and Laboratory Med

**Abstract Submission:**

Thyroid hormones (TH) like thyroxine (T4) and triiodothyronine (T3) exert versatile effects on human metabolism, including the regulation of the basal metabolic rate, heart rate or oxidative phosphorylation. Hyperthyroidism characterized by an excess of TH results in a global disarrangement of metabolic processes. Yet, current clinical marker, including thyroid stimulating hormone (TSH), lack ability for the early diagnosis of (subclinical) hyperthyroidism. To profile the metabolic consequences of TH excess we applied untargeted metabolome and proteome analyses in a human model of experimental thyrotoxicosis. Thyrotoxicosis was induced in sixteen healthy young men using 250µg/day levothyroxine for eight weeks. Plasma was sampled in intervals of four weeks, starting before and ending eight weeks after stopping the application. Protein and metabolite levels were determined using mass spectrometry coupled with liquid chromatography. Mixed-effect linear regression models were used to associate metabolite/protein levels with serum free T4 (FT4) concentrations. Predictive character of a molecule signature for thyrotoxicosis was assessed by a random forest via a two-stage cross-validation procedure, separating training and validation. Surprisingly, we observed versatile molecular alterations in the absence of obvious clinical symptoms. About one fifth of the plasma metabolome and proteome was significantly altered, where the majority of these associations were positive. Besides known TH-related effects on lipid metabolism or the coagulation cascade, a number of novel associations became apparent, like a strong, positive association with  $\gamma$ -glutamyl amino acids or a possible activation of the complement system. A subset of 15 metabolites/proteins attained promising (AUC=0.86) discrimination between thyrotoxicosis and euthyroidism. The use of untargeted OMICS approaches allowed us to reveal novel pathways of TH action and possess ability to identify new molecular signatures, beyond TSH and FT4, for diagnosis and treatment of thyroid disorders.

**Poster #: 388**

**Abstract #: 2117**

**Abstract Title:** GLYCOSPHINGOLIPID ANALYSIS BY UHPLC-MS IN FIBROBLASTS – A FEASIBILITY STUDY

**Authors:** Anne Wehrhan,

**Presenting Author Affiliation:** University Institute of Clinical Chemistry

**Abstract Submission:**

GLYCOSPHINGOLIPID ANALYSIS BY UHPLC-MS IN FIBROBLASTS – A FEASIBILITY STUDY

Anne Wehrhan<sup>1</sup>, Cédric Bovet<sup>1</sup>, Matthias Gautschi<sup>1, 2</sup>, Jean-Marc Nuoffer<sup>1, 2</sup>

<sup>1</sup>University Institute of Clinical Chemistry, Inselspital, Bern University Hospital, Bern, Switzerland

<sup>2</sup>University Children's Hospital, Inselspital, Bern University Hospital, Bern, Switzerland

Sphingolipids are structural components of cellular membrane and important signalling molecules. Genetic defects in the sphingolipid metabolism lead to substrate accumulation and cause severe lysosomal diseases, primarily of the central nervous system and visceral organs (Kolter and Sandhoff 2006). Chemically, sphingolipids are a complex and diverse class of lipids. Lipid profiling by ultra-high performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) allows specific and sensitive detection of sphingolipids and support their characterisation in complex matrices, such as fibroblasts. Because glycosides are liable to in-source fragmentation, the simultaneous analysis of sphingolipids with various degrees of glycosylation is analytically challenging.

We optimized our semi-quantitative UHPLC-MS lipidomics method (adopted from Damen et al. 2014) to characterize the sphingolipid composition in cultured fibroblasts carrying specific enzymatic deficiencies. Analysis of selected sphingolipids (e.g. globosides, gangliosides, ceramides, sulfatides) demonstrate robust chromatographic performance (reproducibility of retention time < 0.05 min) and suitable ionization conditions (positive and negative ion mode) limiting in-source fragmentation of glycosphingolipids and adduct formation. In control fibroblasts a range of sphingolipids (e.g. ceramides, sulfatides, globosides) was successfully detected, along with other more abundant cell lipids, such as glycerophosphocholines.

In a feasibility study patient derived fibroblasts carrying specific enzymatic deficiencies, e.g. from Metachromatic Leukodystrophy, Fabry, Gaucher, and GM2 diseases, are analysed. The capability of the analytical approach to distinguish between the various enzymatic deficiencies and controls will be discussed.

**References:**

Kolter, T., Sandhoff, K.: Sphingolipid metabolism diseases. *Biochimica et Biophysica Acta* (2006) 1758: 2057–2079.

Damen et al.: Enhanced lipid isomer separation in human plasma using reversed-phase UPLC with ion-mobility/high-resolution MS detection. *J. Lipid Res.* (2014) 55:(8) 1772-1783.

**Poster #: 389**

**Abstract #: 2351**

**Abstract Title:** Metabolite identification using Orbitrap based spectral library matching: challenges and insights

**Authors:** Praveen Kumar K,

**Presenting Author Affiliation:** Beaumont health system

**Abstract Submission:**

Metabolite identification using Orbitrap based spectral library matching: challenges and insights.

Praveen Kumar<sup>1</sup>, Ray O. Bahado-Singh<sup>1</sup>, David S. Wishart<sup>2</sup>, Stewart F. Graham<sup>1</sup>

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Untargeted analysis employing Orbitrap based instruments is a popular choice for discovery metabolomics studies to generate biologically meaningful hypothesis. However, one major bottleneck to generate such hypothesis is the unambiguous identification of metabolites, which can be attributed to the lack of Orbitrap metabolite spectras in publicly accessible spectral databases. To address this, an in-house retention time (RT), MS/MS spectral library was created using about 800 metabolite standards kindly provided by Human Metabolite Database (HMDB Alberta, CA). Of the 800 metabolites, 510 were characterized using reverse phase liquid chromatography (LC) coupled to LTQ/Orbitrap Elite in both ESI + and ESI – modes of acquisition. A 20 minute gradient was applied and the spectral data were acquired in data dependent acquisition mode with an isolation width of 1Da. Spectral data of each compound was acquired at three different normalized collisional energies (NCE) – 10, 30 and 70. Human serum extracts were analyzed using the same LC gradient and LTQ/Orbitrap Elite method, which was used for creating the library. The features detected were annotated following the recently proposed quantitative metabolite identification metrics, by matching RT, accurate mass of the parent ion and accurate mass tandem mass spectra. A few challenging cases were observed, where RT and spectral matching were not sufficient to confirm the identity of the compounds. Herein, we describe some examples and potential solutions to support in generating best practices for accurate identification of metabolites and avoid false positives.

**Poster #: 390****Abstract #: 2404****Abstract Title:** Spontaneously hypertensive rats on high-fat diet as a model of metabolic syndrome and impact of antiobesity treatment**Authors:** Helena Pelantová, Martina Bugánová, Blanka Šedivá, Martina Holubová, Marek Kuzma, Lenka Maletínská, Jaroslav Kuneš,**Presenting Author Affiliation:** Institute of Microbiology ASCR**Abstract Submission:**

Metabolic syndrome refers to a cluster of disorders (obesity, diabetes, arterial hypertension and dyslipidemia). Spontaneously hypertensive rats (SHR) on high-fat (HF) diet can serve as a model of metabolic syndrome because HF diet-induced metabolic changes could be expected besides an established hypertension. Prolactin-releasing peptide (PrRP) is anorexigenic neuropeptide with the potential to decrease food intake and ameliorate obesity. The aim of our study was to characterize for the first time the SHR-HF model using NMR-based metabolomics in combination with the standard biochemical parameters and to evaluate the effect of novel PrRP analog on energy balance regulation.

Male SHR rats were for 15 weeks supplied with a HF diet and then for 3 weeks treated with palmitoylated PrRP analog. Pre-processed Carr-Purcell-Meiboom-Gill spectra of urine samples, collected before and after dosing period, were analyzed by Partial least square – discriminant analysis (PLS-DA) and parametric and nonparametric univariate tests to identify metabolites the most contributing to the group discrimination. PLS-DA models display substantial separation of rats on HF and standard diet before antiobesity intervention. Urinary metabolic profiles of obese rats reveal attenuated concentrations of numerous metabolites in aromatic part of spectra, mainly trigonelline, hippurate and phenylacetylglycine, compared to their age-matched lean controls.

Three-week treatment of obese SHR-HF rats with the palmitoylated PrRP analog caused a significant reduction in food intake and body weight, decreased liver weight and plasma leptin levels. NMR metabolomics shows clear discrimination between groups of treated and control SHR-HF rats the separation is based predominantly on changes of taurine, creatinine, 2-oxoglutarate, citrate, and phenylacetylglycine levels. To identify a potential relationship between the urine metabolic composition and pathologies clustered in metabolic syndrome, the metabolite concentrations will be correlated with monitored biochemical parameters.

This work is supported by the Grant Agency of the Czech Republic (Grant No. 13-14105S).

**Poster #: 391**

**Abstract #: 2539**

**Abstract Title:** Metabolic profiling of exhaled breath condensates as potential diagnostic tool for patients with idiopathic pulmonary fibrosis – a pilot study

**Authors:** Barbara Rindlisbacher, Carina Strebel, Sabina Guler, Thomas Geiser, Cédric Bovet, Manuela Funke,

**Presenting Author Affiliation:** Clinical Metabolomics Facility, University Institu

**Abstract Submission:**

Idiopathic pulmonary fibrosis (IPF) is a fast progressing lung disease causing fatal respiratory failure. IPF diagnosis and monitoring remains a challenge as limited indicators for disease progression exist. Sampling of exhaled breath condensate (EBC) holds great potential for diagnosis and monitoring of the disease as the material is easily and non-invasively collected. However, biomarkers found in EBC are difficult to identify due to low metabolite concentrations and methodological limitations. The aim of this study was to identify discriminatory metabolic profiles in EBC between IPF patients and healthy controls (n=10) using ultra-performance liquid chromatography coupled to high-resolution mass spectrometry (UHPLC-MS). Additionally, intra-day and inter-day variability of EBC metabolic profiles were assessed in three subjects. An in-house established bioinformatics pipeline was applied for data processing and analysis. Principal component analysis (PCA) and orthogonal projection to latent structures-discriminant analysis (OPLS-DA) were used to identify discriminative metabolites between IPF and controls. In total, 31 potential metabolites were found to be discriminative between IPF and controls (FDR corrected Mann-Whitney q-value =0.05). Changes up to 5-fold were observed and the majority of the discriminative metabolites (84%) were down-regulated in IPF compared to controls. Even though the results require confirmation in a larger cohort, non-targeted metabolic profiling by UHPLC-MS of EBC exhibits great potential to improve diagnosis and monitoring of IPF patients and therefore deserves further exploration.

**Poster #: 392**

**Abstract #: 2560**

**Abstract Title:** Utility of lung-related biofluids in metabolomics-based lung disease research

**Authors:** Nichole Reisdorph,

**Presenting Author Affiliation:** University of Colorado Anschutz Medical Campus

**Abstract Submission:**

COPD and asthma are multifactorial diseases with complex genetic and environmental factors, making proper diagnosis and treatment a challenge. Our team uses a variety of tools to study COPD and asthma, including metabolomics, systems, and targeted small molecule approaches. Studies utilize plasma, urine, lung biopsies, bronchoalveolar lavage (BAL) fluid, sputum, saliva, exhaled breath condensate (EBC), and nasal brushes/washes. The general utility and relevance of these molecules in the context of lung disease will be discussed. Biofluids were collected as part of various independent studies. Several studies used matched samples as follows: A) plasma vs BAL in humans, B) plasma vs BAL in mice, C) plasma vs lung tissue in mice, and D) EBC vs saliva in humans. Liquid-liquid extraction was performed followed by LC/MS analysis of resulting aqueous and lipid fractions. Mass Profiler Professional and R were utilized to identify differences and overlap in metabolites among sample types. Metabolites were annotated using public and in-house databases identities were confirmed with standards and/or MSn where possible. Targeted analyses for lipid mediators and amino acids were performed as published previously. Approximately 50% overlap was found between mouse and human plasma, over 34% of BAL molecules were also detected in plasma, over 1500 compounds were detected in sputum, over 500 molecules in saliva, and approximately 300 in nasal washes. 400 metabolites were detected in EBC and saliva, of which 77 compounds were specific to EBC. Of the sixteen eicosanoids targeted in EBC, only 8-iso-15R-PGF2a was detected. In contrast, ten of sixteen eicosanoids were detected in saliva. Eighteen out of 31 lipid mediators were discovered in mouse and human plasma thirteen were detected in lung tissue. Small molecule analysis has revealed significant overlap between lung-related biofluids. Several lung-related molecules were also found to be present in plasma. Significant salivary contamination can affect EBC results.

**Poster #: 393**

**Abstract #: 2571**

**Abstract Title:** Non-targeted metabolomics approach to evaluate the effects of a selective COX-2 inhibitor in asthma

**Authors:** Caroline Mathon, Hector Gallart Ayala, Shama Naz, Stacey Reinke, Kameran Daham, Sven-Erik Dahlen, Barbro Dahlen, Craig Wheelock,

**Presenting Author Affiliation:** Karolinska institut

**Abstract Submission:**

Asthma is a chronic respiratory disorder, affecting 300 million individuals worldwide. Symptoms include episodic airway inflammation coupled with reversible obstruction. Prostaglandins are lipid mediators of inflammation that impact the airways, affecting constriction and relaxation. The prostaglandins are generated enzymatically by COX-1 or COX-2 (cyclooxygenase) from arachidonic acid. We evaluated effects of the selective COX-2 inhibitor etoricoxib on: 1) prostaglandin levels in urine, 2) physiological parameters (e.g. lung function), 3) safety in asthmatic subjects, and 4) urine metabolic profiling. Herein, we report the metabolic changes observed in urine.

In this study, 16 nonsmoking subjects with mild atopic asthma, underwent treatment with rising dose inhalation of allergen or methacholine during a control study period, or after 10 to 13 days of treatment with etoricoxib (90 mg/day). Urine samples were collected before the start of allergen bronchoprovocation, and at 1 and 2 hours after the maximum fall in lung function.

Urine samples were diluted based upon the specific gravity, proteins precipitated, and analyzed on four complementary UHPLC-QToF methods combining positive and negative ESI with reversed phase and HILIC chromatography on an Agilent 6550 QToF. The acquisition method was based on three parallel experiments: full scan, all ions fragmentation at 10eV and at 30eV. This approach enables simultaneous untargeted analysis and MS/MS information of all ions (All Ions Fragmentation). HRMS data were processed using a custom database containing retention times and fragmentation information for >700 metabolites.

Data for the HILIC separation in positive ionization identified 75 metabolites. Of these compounds, 14 were significantly altered over time ( $p < 0.05$ ), amongst which, hypoxanthine, tyramine and xanthosine showed a  $p < 0.001$ . These results highlight increased oxidative stress in the urine of asthmatics following challenge. Data analysis of the remaining methods is undergoing and expected to provide increased insight into the metabolic responses associated with COX-2 inhibition in asthma.



**Poster #: 394**

**Abstract #: 2579**

**Abstract Title:** The analysis of extremely polar compounds

**Authors:** SCOTT HARRISON, Andrew Chetwynd, Donna O'Neil, Giovanni Rodriguez Blanco,

**Presenting Author Affiliation:** University of Birmingham

**Abstract Submission:**

Introduction: Mass spectrometry analysis of biologically important metabolites including adenosine phosphates (ATP, ADP, AMP), NADH, NADPH polyamines, purines and pyrimidines still remains as a challenge in metabolomics experiments. These compounds exhibit a high polarity so that conventional reversed phase ultra-high performance chromatography (RP-UHPLC) methods using a C18 column cannot separate these molecules as they are poorly retained if at all. A search for alternative column phases such as polar C18 proved unfruitful and this has left HILIC, Cyano and aqueous normal phase (ANP) as viable options to consider. In this paper we compare these 3 methods and assess their efficacy for the analysis of extremely polar compounds.

Results: Aqueous C18 columns did not give sufficient retention of phosphate containing compounds. In all 3 methods proved to be problematic to balance the dilution solvent and in ANP the triphosphates could not be detected. Although the peak shape for some of the compounds was not ideal, being somewhat broad, these methods proved to be the most practical available option. Mass spectrometry analysis of very polar compounds in different matrices such as urine, plasma and cell line extracts will be discussed. With peak shape and retention time stability being considered

**Poster #: 395**

**Abstract #: 2584**

**Abstract Title:** Consortium for METabolomics Studies (COMETS): Leveraging Resources to Accelerate Scientific Discovery

**Authors:** Marc Gunter, Steven Moore, Cornelia Ulrich, Rachael Stolzenberg-Solomon, Elizabeth Poole, Marinella Temporsa, Mukesh Verma, Demetrius Albanes, Eric Boerwinkle, Juan Casas, Clary Clish, Robert Gerszten, Bing Yu, Tamara Harris, D

**Presenting Author Affiliation:** International Agency for Reserach on Cancer

**Abstract Submission:**

Metabolomics is a systems approach to the biology of health and disease and an ‘-omics’ discipline that measures small metabolites representing end products of a variety of metabolic and cellular processes as reflected in available biological specimens (e.g. blood, urine, saliva, feces and tissue). As an emerging approach used for epidemiologic and clinical studies, metabolomics has the potential to improve disease risk assessment, screening, diagnosis, prognosis and predictive response to therapy, as well as provide disease mechanistic insight and help to establish criteria for causation. The number of publications of epidemiological studies that use metabolomics technology has rapidly increased over the past 10 years, including application to a wide variety of exposures and disease states.

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 25 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 follow participants for a range of outcomes and perform metabolomic profiling of individuals. COMETS aims to facilitate an open exchange of ideas, knowledge, and results to accelerate a shared goal of identifying metabolomic profiles associated with chronic disease phenotypes (e.g. heart disease, diabetes, cancer). Here we describe the COMETS structure, including preliminary descriptive data, which aims to advance the use and impact of metabolite profiling in population-based research.

**Poster #: 396**

**Abstract #: 2595**

**Abstract Title:** Staphylococcus aureus Mediated Cell Death in Keratinocytes

**Authors:** Sage Schiller,

**Presenting Author Affiliation:** Montana State University

**Abstract Submission:**

Staphylococcus aureus a Gram-positive bacteria, is a facultative anaerobe which causes opportunistic infections in the immunocompromised. S. aureus can be cultured as two different phenotypes: biofilm and planktonic. In order to grow into a biofilm state there needs to be adhesion of cells to a surface, and adhesion between cells to form multilayered clusters of cells. The biofilms are surrounded by a matrix made up of exopolysaccharides, nucleic acids, and proteins and are significantly more resistant to both therapeutic treatment and the endogenous immune system when compared to planktonic cultures. Previously, it has been observed that small molecules secreted by S. aureus induce programmed cell death in keratinocytes through distinct mechanisms. We use PCR (polymerase chain reaction) array mapping of the human programmed cell death signal transduction pathways to determine how secreted factors from S. aureus biofilms are killing the keratinocytes. By evaluating the pathways, we can determine the difference in programmed cell death of keratinocytes between the S. aureus biofilm and planktonic states. Additional experiments include metabolomics profiling of all small molecule metabolites secreted by the biofilm and planktonic phenotype of S. aureus cultures. By correlating how differences in small molecule metabolite profiles determine the biochemical regulation of programmed cell death in host cells, we can further understand how metabolism effects the host innate immune response to wound colonization by opportunistic pathogens such S. aureus.

**Poster #: 397**

**Abstract #: 2076**

**Abstract Title:** UPLC-MS/MS Analysis of Glucosylceramide and Galactosylceramide Isoforms in Parkinson's Disease Brain Tissues

**Authors:** Michel Boutin, Christiane Auray-Blais, John Shacka,

**Presenting Author Affiliation:** Universite de Sherbrooke

**Abstract Submission:**

Deficiency in glucocerebrosidase (GCase) enzymatic activity is a major risk factor for Parkinson's disease, and is suspected to promote alpha-synuclein pathologies. GCase is involved in the lysosomal recycling of glucosylceramide (GluCer) by the cleavage of its beta-glucosidic linkage. We therefore evaluated GluCer in brain tissues as a Parkinson's disease biomarker. GluCer has different isoforms owing to different fatty acid chains attached to its sphingosine moiety. The main challenge for the analysis of GluCer isoforms was to chromatographically separate them from their galactosylceramide (GalCer) structural isomers which are differentiated only by the axial position of one hydroxyl group on the sugar moiety. Human brain tissue samples were homogenized using a bead mill (Bead Ruptor 12, Omni). Brain homogenates were extracted using a hydrophilic-lipophilic balance (HLB) solid phase extraction cartridge (Oasis, Waters Corp.). Samples were separated using a normal phase column (Hilic) on an Acquity I-Class system (Waters). A Xevo TQ-S tandem mass spectrometer (Waters) was used in the Multiple Reaction Monitoring (MRM) mode for detection. Five GluCer isoforms (C18:0, C20:0, C22:0, C24:1, C24:0) and their GalCer counterparts were monitored with limits of detection ranging from 0.4 to 1.1 nmol/g brain. Synthetic GalCer(C15:0) and GluCer(C16:0)D3 were respectively used for the calibration curve and the internal standard. Temporal cortex brain tissue samples from 26 Parkinson's disease patients at different stages of the disease (IIa, III, and IV) were analyzed and compared to 12 controls and 6 patients with incidental Lewy Body Disorders. No significant GluCer concentration differences were observed between the 5 sample groups. However, when normalized with their GalCer counterparts, the GluCer isoforms showed an increasing trend with PD severity.

**Poster #: 398**

**Abstract #: 2093**

**Abstract Title:** Investigation of the Stability of Dried Blood and Urine Spots for Untargeted Metabolomic Applications of Healthy Ageing

**Authors:** Elliott Palmer,

**Presenting Author Affiliation:** University of Birmingham

**Abstract Submission:**

Introduction: Dried Blood and Urine Spots (DBS and DUS) represent an alternative strategy for sample collection in small and large-scale untargeted metabolomic studies and represent an attractive scenario to many researchers. The ability to collect samples away from the clinical environment by individuals not trained in phlebotomy and without the requirement of rapid sample processing in the laboratory are all advantages for healthcare systems and the individuals being studied.

Objectives: There is a lack of published research in relation to the collection and storage of DBS and DUS for untargeted metabolomics studies [1,2]. One of the major issues concerns the long-term stability of samples and the appropriate storage conditions. Studies targeting amino acids have shown instability for samples stored at room temperature [3]. However, the study of different classes of metabolites investigated in untargeted metabolomics research has not been thoroughly investigated. As part of a long-term stability study, here we report stability data for 28 days for DBS and DUS to determine short-term optimal storage conditions.

Methods: 20µL aliquots of whole blood and urine from rats were deposited onto Whatman Protein Saver Cards #903. Cards were stored at room temperature, 4°C or -20°C and extracted applying 80/20 methanol/water at days 1, 3, 7, 14 & 28. Counterpart biofluids were also stored for equivalent times and temperatures and extracted. Samples were analysed by a chip based nano-electrospray direct infusion mass spectrometry method.

Results: The stability of data is dependent on the storage temperature and time and metabolite class-specific good and poor stability will be described.

[1] Michopoulos, et al. J. Proteome Res. 2010, 9, 3328–3334 [2] Koulman et al., Metabolomics. 2014, 10, 1–8 [3] Prentice et al. Bioanalysis. 2013, 5, 1507–1514

**Poster #: 399**

**Abstract #: 2121**

**Abstract Title:** Metabolic perturbations associated with exercise to exhaustion and the influence of cherry juice to reduce oxidative stress during exercise

**Authors:** Giovanny Rodriguez Blanco, Riccardo Di Guida, Elliott Palmer, Andrew Chetwynd, Emily Bailey, Matthew Soden, Scott Harrison, Sarah Aldred, Warwick Dunn,

**Presenting Author Affiliation:** Phenome Centre Birmingham, University of Birmingham

**Abstract Submission:**

**Introduction:** Exercise is an important lifestyle choice to maintain a healthy BMI, reduce the risk of chronic diseases and to maintain an appropriate level of musculoskeletal health. Exercise causes a transient increase in reactive radical species, which is essential for adaptation. However, under certain circumstances radical species may create a situation of oxidative stress. The use of supplements (for example, cherry juice), which contain high levels of antioxidant may benefit some individuals undertaking exercise by reducing levels of reactive oxygen species that increase following exercise or during the recovery period.

**Objectives and Methods:** The objective was to investigate the metabolic changes associated with different intensity exercise bouts, and the effect of cherry juice supplementation on metabolic perturbations associated with exercise. Eight healthy subjects who performed greater than 5 hours of cycling per week were investigated in a crossover study involving daily cherry juice supplementation versus placebo. Blood samples were collected during an exhaustive exercise bout, and a time-trial, undertaken on a cycle ergometer after 7 and 8 days of supplementation or 7 and 8 days of placebo. Blood plasma samples were analysed applying a UHPLC-MS non-targeted metabolomics approach. Following data pre-processing with XCMS, the data were analysed applying univariate and multivariate analysis methods.

**Results and conclusions:** As expected, the largest changes in the plasma metabolomes were observed at different intensities of exercise with carbohydrates, purines, acyl carnitines and fatty acids all showing significant differences. Following removal of all metabolites present in cherry juice, cherry juice had a smaller but distinct influence on metabolic changes observed which implies that cherry juice has an impact on metabolism. Further work is required to ascertain whether this change is beneficial in reducing oxidative stress.

**Poster #: 400**

**Abstract #: 2146**

**Abstract Title:** LYSOPHOSPHATIDYLCHOLINES TO PHOSPHATIDYLCHOLINES RATIO PREDICTS ADVANCED KNEE OSTEOARTHRITIS

**Authors:** weidong zhang, guang sun, Dawn Aitken, Sergei Likhodii, ming liu, Glynn Martin, Andrew Furey, Ed Randell, Proton Rahman, Graeme Jones, guangju zhai,

**Presenting Author Affiliation:** memorial university of newfoundland

**Abstract Submission:**

**Objectives:** To identify novel biomarker(s) for predicting advanced knee OA.

**Methods:** Study participants were derived from the NFOAS and the TASOAC studies. All knee OA cases were patients who underwent total knee replacement (TKR) due to primary OA. Metabolic profiling was performed on fasting plasma using a targeted metabolomics approach. 4,018 plasma metabolite ratios that were highly correlated with that in synovial fluid in our previous study were calculated as surrogates for joint metabolism.

**Results:** The discovery cohort included 64 TKR cases and 45 controls and the replication cohorts included a cross sectional cohort of 72 TKR cases and 76 controls and a longitudinal cohort of 158 subjects, of whom 36 underwent TKR during the 10-year follow-up period. We confirmed the previous reported association of the branched chain amino acids

to histidine ratio with advanced knee OA ( $p=9.3 \times 10^{-7}$ ) and identified a novel metabolic marker - lysoPCs to PCs ratio -that was associated with advanced knee OA ( $p=1.5 \times 10^{-7}$ ) after adjustment for age, sex, and BMI. When the subjects of the longitudinal cohort were categorized into two groups based on the optimal cutoff of 0.09 of the ratio, we found the

subjects with the ratio  $\geq 0.09$  were 2.3 times more likely to undergo TKR than those with the ratio  $<0.09$  during the 10-year follow-up (95% CI: 1.2-4.3,  $p=0.02$ ).

**Conclusions:** We identified the ratio of lysoPCs to PCs as a novel metabolic marker for predicting advanced knee OA. Further studies are required to examine whether this ratio can

**Poster #: 401**

**Abstract #: 2182**

**Abstract Title:** Metabolome changes during differentiation of induced pluripotent stem (iPS) cell into renal lineages

**Authors:** Anna Artati, Anja Wilmes, Alexander Cecil, Paul Jennings, Jerzy Adamski,

**Presenting Author Affiliation:** Helmholtz Zentrum München

**Abstract Submission:**

At present drug development is hampered by limits in pre-clinical validation process in animal cells or animal model. Human material representing established cell lines derived from heart, brain and pancreatic  $\beta$ -cells has major drawback in missing tissue-specific phenotypes. Recently stem cells which are able to self-renew and are pluripotent, offer a new potential in drug development. They are able to differentiate to any cell type in the human body. Methods of reprogramming ordinary adult cells to create iPS cells were made recently available. In this study, non-targeted metabolomics is used to monitor differentiation of EU consortiums StemBANCC iPS cells into renal lineage whereas biostatistics approaches are applied to identify possible biomarkers of the differentiation steps.

Cells were grown on a 6-well plate format and supernatants were collected (24 h after feeding) on day 0 (undifferentiated iPS cells), days 1, 2, 3, 6, 10, 14, 18 and 21 for metabolomics analysis. In addition the differentiated human proximal tubular cell line RPTEC/TERT1 was included. Detection and relative quantification of the metabolites was done using UHPLC-MS/MS. The MS2 data was matched to Metabolon's database library for metabolite identification.

Metabolome analyses can illustrate different stages of stem cell differentiation. Metabolites detected in different time points during cell differentiation showed some separate clusters, i.e. samples of day 0, samples of day 1 to day 3 and samples of day 6 to day 21 of the differentiation. Samples of the day 21 of differentiation are clustered to the proximal tubular cell line. Some metabolites involved in TCA cycle and amino acid metabolism contribute to the clustering. Biomarker derived from these analyses can be used to monitor process and implement quality assurance measures.



**Poster #: 402**

**Abstract #: 2255**

**Abstract Title:** CYP3A ACTIVITY AMONG SUBJECTS WITH DIFFERENT CYP2C19 GENOTYPES: GC-MS BASED QUANTITATION OF ENDOGENOUS METABOLIC MARKERS

**Authors:** Andrew Kim, Jaeseong Oh, Bora Kim, Jieon Lee, SeungHwan Lee, Kyung-Sang Yu, In-Jin Jang, Joo-Youn Cho,

**Presenting Author Affiliation:** Department of Clinical Pharmacology and Therapeuti

**Abstract Submission:**

Cytochromes P450 (CYP) 3A subfamily is the most abundant drug-metabolizing enzyme group in the human liver and it is involved in hydroxylation of many different kinds of drugs and endogenous substrates such as steroids. Additionally, CYP2C19 enzyme also has its role in metabolizing specific drugs. However, CYP2C19 enzyme has higher frequency of genetic polymorphism in Asians than Caucasians, which can result in different therapeutic efficacy among subjects with different CYP2C19 genotypes. In previous studies, concentration of fourteen endogenous steroids were suggested to be the endogenous metabolic markers of CYP3A activity. The aim of this study was to measure the CYP3A activity in subjects with different CYP2C19 genotypes by quantitation of urinary and plasma steroids concentration as endogenous metabolic markers of CYP3A activity. A total of twenty-four healthy men were recruited based on their CYP2C19 genotypes: eight extensive metabolizers (EMs, \*1/\*1), eight intermediate metabolizers (IMs, \*1/\*2 or \*1/\*3), and eight poor metabolizers (PMs, \*2/\*2, \*2/\*3 or \*3/\*3). 12h interval urine (n=24) and plasma samples (n=20) to phenotype CYP3A activity were collected from each subject and endogenous metabolic markers were quantitated with gas chromatography coupled with triple quadrupole mass spectrometry. As a result, the fold changes of urinary 6 $\beta$ -hydroxycortisol/cortisol, 6 $\beta$ -hydroxycortisone/cortisone, 11-deoxycortisol/cortisol, 11 $\beta$ -hydroxyandrostenedione/androstenedione ratio and plasma concentration of 4 $\beta$ -hydroxycholesterol in PMs were 2.4, 3.3, 1.7, 1.6, and 2.4 respectively compared to those of EMs. In conclusion, the endogenous metabolic markers of CYP3A activity were significantly higher in CYP2C19 PMs compared to those of EMs. This result suggests that the subjects with lack of CYP2C19 enzyme function may have higher CYP3A enzyme activity.

**Poster #: 403**

**Abstract #: 2277**

**Abstract Title:** A simple method for the diagnosis of bile acids malabsorption: quantification of 7 $\alpha$ -hydroxy-4-cholesten-3-one

**Authors:** Jean-Christophe Prost,

**Presenting Author Affiliation:** Inselspital

**Abstract Submission:**

Bile acids malabsorption is encountered in numerous gastrointestinal pathologies and is a good example of treatable cause of watery diarrhea after ileal resection. Robust diagnostic methods to detect malabsorption are not routinely used in practice due to cost, logistics or laboratory equipment limitations. The current available test for the measurement of bile acids malabsorption is the selenium homocholic acid taurine (SeHCAT) test, an expensive and complex nuclear medicine method. An alternative approach could be the quantitative analysis of 7 $\alpha$ -hydroxy-4-cholesten-3-one (7 $\alpha$ OHC), an intermediate metabolite in the synthesis of bile acids from cholesterol. Methods using high-performance liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS) have demonstrated good sensitivities in the low ng/mL concentrations for 7 $\alpha$ OHC. However, these methods usually used time-consuming sample preparation (liquid-liquid (LLE) or solid phase (SPE) extraction) and long analysis time (typically 14-50 minutes).

For the routine measurements of 7 $\alpha$ OHC in large patient's sets, a simple and faster protocol would be beneficial. Therefore, we developed and validated an ultra-high performance LC-MS/MS (UHPLC-MS/MS) assay to efficiently quantify 7 $\alpha$ OHC in human serum. The analyte was extracted using immobilized coating extraction technology (Tecan, 96-well plate format), an absorptive extraction coating for principally non-polar molecules. The quantification of 7 $\alpha$ OHC was then performed over the concentration range of 5-300 ng/mL ( $R^2 = 0.9977$ ) in a 6 min run time by reversed-phase chromatography coupled to a triple quadrupole mass spectrometer (Acquity I-Class UPLC coupled to Xevo TQ-S, Waters). Accuracy and precision were less than 15% for all QCs (low, middle, high). Our laboratory is now offering this assay for the verification of the bile acids malabsorption diagnose by measuring the decrease in concentration of 7 $\alpha$ OHC after treatment.

**Poster #: 404****Abstract #: 2389**

**Abstract Title:** Early changes in acylcarnitine profile in blood and urine of obese patients are reversed 90 days post Roux-en-Y gastric bypass surgery

**Authors:** Thais Lima, Carina Fernandez, José Rubens Arnoni Junior, José Araújo Jr, Cinthia Taglieri, Hannelore Daniel, Jarlei Fiamoncini,

**Presenting Author Affiliation:** University of Sao Paulo

**Abstract Submission:**

Obesity severely affects human health and Roux-en-Y gastric bypass (RYGB) is an effective method to achieve sustained weight loss and diabetes remission. In addition, bariatric surgery patients may provide important data regarding metabolic adaptations due to remarkable changes in food intake, with a view to clinical biomarker development. Therefore, we employed a metabolomics approach in 20 obese patients who underwent RYGB. Fasting plasma samples were collected before, 15 and 90 days after surgery in order to assess clinical chemistry markers. In the same days, total blood samples (dry blood spots) and urine were collected and subjected to acylcarnitine and phospholipids profiling using LC-MS/MS. As expected, RYGB resulted in significant weight loss with normalization of biochemical parameters (blood glucose, cholesterol fractions and triglycerides). By analyzing 28 species of acylcarnitines in patients blood, we found an increase in the concentration of long [C18:1 (33%), C16 (12%)], medium [C10 (48%), C8 (43%)] and short chain (C2, 45%) acylcarnitines early after surgery. C4-OH carnitine, which can be derived from the CoA ester of the ketone body D-3-hydroxybutyrate or the FAO intermediate L-3-hydroxybutyryl-CoA, increased 7 times. However, this effect was lost 90 days after surgery, indicating metabolic adaptation of the patients. The concentration of acylcarnitines related to amino acid catabolism (C3, C5) decreased throughout the period evaluated, indicating that protein catabolism is inhibited in this scenario. We found an increase C4-OH (130%), C5-DC (31%) and C6-DC (90%) in patients' urine 15 days after surgery, but only C6-DC levels correlated with blood concentration. As observed in blood, values returned to basal after 90 days. Our data indicate that metabolic adaptation is still in course 90 days after bariatric surgery.

**Poster #: 405**

**Abstract #: 2490**

**Abstract Title:** Evolution of Biochemical Markers in Plasma from Newborns with Hypoxic-Ischemic Encephalopathy (HIE) during Hypothermia Treatment

**Authors:** Ángel Sánchez-Illana, Antonio Núñez-Ramiro, María Cernada, Anna Parra, Vicent Yusà, Julia Kuligowski, Máximo Vento,

**Presenting Author Affiliation:** Neonatal Research Group. IIS La Fe

**Abstract Submission:**

Hypoxic-ischemic encephalopathy (HIE) as a result of perinatal asphyxia is a major cause of neurologic disabilities and mortality in the term neonate. Therapeutic hypothermia (TH) is standard of care for infants with HIE, involving a core temperature reduction to 33-34.5 °C for 72 h. However, the effect of TH on the metabolism of neonates has not yet been explored in depth.

In this particular context, ranges of eight metabolites involved in central metabolic pathways including lactate, pyruvate, metabolites from the Krebs cycle as well as ketone bodies were determined in plasma samples from newborns with HIE undergoing hypothermia treatment (N=85). Blood samples were withdrawn from umbilical cord blood and 24, 48 and 72 h after initiation of TH. Levels were compared to a control group comprising samples from healthy term newborns at 48 h of age.

The GC-MS based analytical method was successfully validated following the recommendations of the US Food and Drug Administration (FDA) guidelines for bioanalytical method validation and allowed the simultaneous quantification of the studied metabolites employing a sample volume of only 50 µL enabling serial determinations from small volume blood samples.

In hypothermic conditions, pyruvate and lactate in cord blood were elevated in comparison to controls ( $p<0.01$ ). This significant alteration in pyruvate levels persists over 72h. However, in the case of lactate, this significant increase was not observed after 48 h. In both compounds, significant differences between cord blood, 24, 48 and 72 h were found ( $p<0.05$ ) indicating a tendency towards normal levels. As for the metabolites from the Krebs cycle, these remained unchanged. Acetoacetate and  $\beta$ -hydroxybutyrate levels decreased significantly during TH ( $p<0.01$ ), reflecting differences in the nutritional status.

**Poster #: 406**

**Abstract #: 2508**

**Abstract Title:** Global metabolite and lipid profiling in bone tissue from aging mouse model associated with osteoporosis

**Authors:** Miso Nam, Sunhee Jung, Do Hyun Ryu, Geum-Sook Hwang,

**Presenting Author Affiliation:** Sungkyunkwan University

**Abstract Submission:**

Osteoporosis is one of the most common diseases associated with age and has characteristic of the increased fracture risk by the bone mineral loss. Many studies have been underway to identify the biological pathways for age-related osteoporosis. However, there is little known about age related changes in bone metabolism.

In this study, we applied global profiling to bone tissue of young and aged male mice to determine age-related metabolic changes in bone metabolism. The C57BL/6J mice were divided into two groups according to age young and aged mouse groups. Ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry (UPLC/QTOF MS) analysis was performed for global polar and lipid profiling of bone tissue to find metabolic differences between young and aged mice.

In principal component analysis (PCA) and orthogonal partial-least-squares discriminant analysis (OPLS-DA) models showed clear differentiation between young and aged groups, and significant difference in a few polar and lipid metabolites were observed between two groups. Metabolites associated with reactive oxygen species (ROS) metabolism were increased in bone tissue of aged mice. Levels of sphingolipids, di- and triacylglycerol were decreased in aged mice. In addition, levels of monoacylglycerol and several phospholipids were increased in aged mice. Here, we first performed global polar and lipid profiling in bone tissue of aging mouse and suggested age-related metabolic changes in bone

**Poster #: 407****Abstract #: 2514**

**Abstract Title:** The maternal hair metabolome in pregnancy predicts subsequent aberrant neurodevelopment in infants

**Authors:** Beatrix Jones, Morgan Han, Thibaut Delplancke, Elizabeth McKenzie, Chong Yap-Seng, Seang-Mei Saw, Kenneth Kwek, Michael MEANEY, Peter Gluckman, Anne Rifkin-Graboi, Philip Baker,

**Presenting Author Affiliation:** Massey University

**Abstract Submission:**

**Background:** 10%-20% of individuals worldwide have a neurodevelopmental disability. The fetus undergoes intense neurodevelopment in utero, however, neurodevelopment deviation in the perinatal and infancy period is often subtle and highly specified and expensive neuroimaging techniques are often considered necessary for detection. Our hypothesis is that the maternal hair metabolome may predict aberrant fetal neurodevelopment, and thus facilitate early identification of babies who will benefit most from intervention.

**Methods:** We utilized maternal hair samples obtained at 26-28 weeks gestation in the comprehensive longitudinal GUSTO cohort study of pregnancy, infancy and childhood in Singapore (1011 infant-mother dyads). Neurocognitive assessment of infants included the Bayley III Scales of Infant Development (BSID-III) at 24 months. GC-MS was performed, as previously (Sulek K et al. Theranostics 2014 4:953). Data was deconvoluted using AMDIS (<http://www.amdis.net/>) metabolite identification and relative quantitation used in-house R based software and MS library: 182 metabolites were detected.

**Results:** There were strong associations between metabolites and BSID-III psycholinguistic, but not motor, outcomes.

- Cognitive - univariate analysis: 21 discriminatory metabolites (p 0.001-0.05, q 0.09-0.15), multivariate regression, cognizant of ethnicity and maternal age: 13 discriminatory metabolites (p  $5.7 \times 10^{-5}$ -0.04)
- Receptive language – univariate: 20 discriminatory metabolites (p 0.002-0.05, q 0.24-0.27), multivariate: 14 discriminatory metabolites (p  $7.3 \times 10^{-5}$ -0.04)
- Expressive language - univariate: 18 discriminatory metabolites (p 0.002-0.05, q 0.17-0.24), multivariate: 3 discriminatory metabolites (p  $1.3 \times 10^{-3}$ -0.02)

The majority of discriminatory metabolites were fatty acids or amino acids

**Conclusion:** Our hypothesis that multiple metabolite biomarkers in maternal hair would reflect the complex and multiple etiologies associated with aberrant fetal neurodevelopment, and identify babies that subsequently demonstrate neurodevelopmental impairment has been confirmed – for cognition and language but not motor development. Several metabolites are significant in each model, suggesting multiple metabolic pathways with associations to cognition and language development, rather than a

single pathway.

**Poster #: 408**

**Abstract #: 2543**

**Abstract Title:** Exploring Changes in Primary Metabolites in Alzheimer's Disease using Targeted LC-MS/MS

**Authors:** Nicola Gray, Min Kim, Chris Titman, Cristina Legido-Quigley,

**Presenting Author Affiliation:** Shimadzu UK

#### **Abstract Submission:**

##### Introduction

High-throughput and unbiased global profiling metabolomic approaches provide a suitable platform to analyse variations in metabolite levels, offering insights into mechanisms of disease through the discovery of novel biomarkers. Targeted approaches, however, offer a more specific, sensitive and quantitative measure for selected compounds. Here we investigate biochemical changes in metabolic processes and enzyme kinetics at the cellular level in patients with Alzheimer's disease using a targeted LC-MS/MS method for primary metabolites in human plasma.

##### Methods

Plasma samples were prepared using a two-phase extraction with methyl tert-butyl ether (MTBE) and methanol/water. The methanol/water phase was injected directly and a pentafluorophenylpropyl (PFPP) column was used to analyse 97 primary metabolites simultaneously, including amino acids, organic acids, nucleotides, nucleosides and co-enzymes. LC-MS/MS analysis was performed on a Shimadzu LCMS-8060 Triple Quadrupole mass spectrometer operated in positive and negative electrospray (ESI) mode using fast polarity switching.

##### Preliminary Results

Human plasma samples from Alzheimer's patients were investigated and compared with healthy age- and sex-matched controls using a targeted LC-MS/MS approach for the analysis of 97 primary metabolites. Unsupervised principal components analysis (PCA) showed that plasma metabolite profiles from disease and control subjects separated and pooled QC samples clustered together indicating good analytical reproducibility. Supervised orthogonal projection to latent structures discriminant analysis (OPLS-DA) was performed between disease and control groups to identify analytes responsible for group differentiation and subsequent filtering based on significance highlights potential pathways affected.

This targeted approach focused on primary metabolites provides a sensitive and selective way of exploring biochemical changes leading to Alzheimer's disease, offering insights into the complex events involved which may play an important role in detection and novel treatment approaches.



**Poster #: 409**

**Abstract #: 2586**

**Abstract Title:** Urinary <sup>1</sup>H-NMR-based metabolic profiling of children with NAFLD undergoing VSL#3 treatment

**Authors:** Federico Marini, Alfredo Miccheli, Giorgio Capuani, Alberta Tomassini, Giulia Pratico, Anna Alisi, Lorenza Putignani, Valerio Nobili,

**Presenting Author Affiliation:** University of Rome "La Sapienza"

**Abstract Submission:**

Nowadays, non-alcoholic fatty liver disease (NAFLD) is one of the most common chronic liver diseases in children. It reaches a prevalence of 13% in the general pediatric population and up to 80% in obese children. The development of NAFLD and its progression to non-alcoholic steatohepatitis (NASH) are complex processes linked to various and not fully understood genetic and environmental factors anyway, different authors report that the interaction between the liver and gut seem to play a key role. In this context, the potential beneficial effects of probiotics, prebiotics and synbiotics have been confirmed in several experimental studies. In particular, our recent clinical trial demonstrated that dietary and VSL#3-based interventions may improve fatty liver by ultrasound and body mass index (BMI) after 4 months. However, as it is impracticable to extensively monitor response to therapy or treatment by liver biopsy, aim of the present study was to identify a panel of potential non-invasive metabolic biomarkers by a urinary metabolic profiling. Accordingly, a urinary metabolic profiling approach was investigated on a group of 31 pediatric NAFLD patients, enrolled in a VSL#3 RCT, employing high-resolution proton NMR (<sup>1</sup>H-NMR) spectroscopy in combination with multivariate data analysis. The NAFLD urinary metabolic profiles were interpreted in term of clinical patient, treatment and chronology pattern correlations and it was found that VSL#3 treatment induced changes in NAFLD urinary metabolic phenotype mainly represented by the host amino acid metabolism (i.e., valine, tyrosine, 3-amino-isobutyrate or β-aminoisobutyric acid [BAIBA]), nucleic acid degradation (pseudouridine [psi]), creatinine metabolism (methylguanidine [MG]) and in the gut microbial amino acid (valine) catabolism (as represented by 2-hydroxyisobutyrate [2-HIB]).

**Poster #: 410****Abstract #: 2020****Abstract Title:** The metabolic regulation by sex hormones: A longitudinal metabolomics study in transgender persons**Authors:** Cornelia Prehn, Alexander Cecil, Tao Xu, Yasmin Roepke, Johannes Fuss, Rui Wang-Sattler, Günter K. Stalla, Guy T'Sjoen, Matthias Auer, Jerzy Adamski,**Presenting Author Affiliation:** Helmholtz Zentrum München, IEG/GAC**Abstract Submission:**

Sex steroid hormones exert a wide range of effects on metabolism. Applying high doses of testosterone to natal females and high doses of estradiol to natal males as cross-sex hormone treatment (CSH) allows study of such effects in a cross-gender setting. Beside sex reassignment surgery, CSH is crucial to acquire the secondary sex characteristics of the desired gender.

As has been shown before, transwomen (male-to-female-transsexuals) seem to have an increased cardiovascular risk despite favorable changes in classical cardiovascular risk factors, which could be shown here by targeted metabolomics.

Targeted metabolomics measurements were performed on serum of fasting transmen (females-to-male transsexuals) and transwomen at baseline and following 12 months of CSH (N = 20/group). This approach was based on ESI-LC-MS/MS measurements by AbsoluteIDQ p180 kit (BIOCRATES AG). RandomForest analysis was applied to detect metabolites of highest interest for grouping of transwomen and transmen patients before and after initiation of HRT. Principal component analysis (PCA) was performed to check whether group differentiation is achievable according to these variables identified by RandomForest and to check if changes in metabolite levels can be explained by a priori gender differences.

CSH for 12 months resulted in significant changes in terms of body composition as well as levels of sex hormones and blood lipids in both groups in approximation of the phenotypic target sex. PCA predicted grouping of individuals. It is determined mostly by the citrulline/arginine-ratio, the total fat and total lean mass of the individual as well as the levels of the amino acids (AA) lysine, alanine and asymmetric dimethylarginine (ADMA). The changes in testosterone and estradiol levels and subsequent changes in body composition play only a minor role in these PCAs.

In this first study we could show that CSH induces several changes in serum metabolites in transwomen and transmen.

**Poster #: 411**

**Abstract #: 2080**

**Abstract Title:** Novel Biomarkers Associated with Disease Manifestations in a Large Taiwanese Fabry Patient Cohort

**Authors:** Christiane Auray-Blais, Pamela Lavoie, Michel Boutin, Huang Chun-Kai, Hsu Ting-Rong, Dau-Ming Niu,

**Presenting Author Affiliation:** Universite de Sherbrooke

**Abstract Submission:**

Fabry disease is an X-linked lysosomal storage disorder caused by a defect in  $\alpha$ -galactosidase A enzyme activity which leads to accumulation of glycosphingolipids, mainly globotriaosylceramide (Gb3) and globotriaosylsphingosine (lyso-Gb3) in biological fluids, vascular endothelium, heart, and kidneys. A late-onset cardiac phenotype associated with the mutation IVS4 + 919G>A (IVS4) was found to be prevalent in the Taiwanese population. A newborn screening program in Taipei established the prevalence at 1:1600 males and 1:800 females. The objective of this research project was to determine the relationship between clinical manifestations of the disease and Fabry biomarkers such as Gb3 and lyso-Gb3 and related analogues. Fabry biomarkers were analyzed in urine and plasma samples from 191 adult and pediatric Fabry patients carrying the IVS4 mutation. Lyso-Gb3 and related analogues were analyzed in urine and in plasma, while Gb3 was analyzed in urine only, using fully validated tandem mass spectrometry methodologies developed by our group. Normal values were established. Statistical analyses were performed using IBM SPSS Statistics version 22. Multiple regression analyses were used to investigate the association between biomarker levels and left ventricular mass index (LVMI) and the Mainz Severity Score Index (MSSI). Gender and age were taken into account throughout the statistical analyses. In this large cohort of Fabry patients, our results show that plasma lyso-Gb3 levels, and urinary analogue levels of lyso-Gb3 at (+16), (+34), and (+50) are positively associated with the LVMI, and/or the MSSI. Lyso-Gb3 and related analogues might be important biomarkers for patients with the IVS4 mutation, considering the observed association between these biomarkers and clinical manifestations. A longitudinal study of these biomarkers in children with the IVS4 late-onset cardiac variant mutation would be important to determine if the clinical severity in adulthood might be predicted by the elevation of urinary and plasma Fabry biomarkers at a young age

**Poster #: 412****Abstract #: 2150****Abstract Title:** Metabolomic state as early indicator of organ improvement in septic shock patients: feasibility study for small data sample**Authors:** Roberta Pastorelli, Alice Cambiaghi, Laura Brunelli, Manuela Ferrario, Karim Bendjelid, Bernardo Bollen Pinto,**Presenting Author Affiliation:** IRCCS\_Istituto di Ricerche Farmacologiche Mario Ne**Abstract Submission:**

Septic shock is a life threatening organ dysfunction caused by a dysregulated host response to infection. The outcome is still poor and current therapies are targeted to reduce symptoms only. So far the Sequential Organ Failure Assessment (SOFA) score is commonly used in clinics for risk stratification and mortality prediction.

This pilot study aimed at investigating whether circulating metabolites can be associated with organ dysfunction index and can shed light on the biochemical pathways early involved so to provide the basis for prompted target therapy. This work is part of our ongoing study ShockOmics (NCT02141607), a multiscale approach to define early markers signatures in different shocks for the delivery of new therapies.

This explorative analysis focused on 30 septic shock patients for which 131 metabolites concentrations were measured at ICU admission (T1, acute-phase) and 48 hours after (T2, post-resuscitation) by a targeted mass spectrometry-based quantitative metabolomic approach using the Biocrates platform (AbsoluteIDQ-p180kit) coupled to Triple-Quad 5500 LC-MS/MS system.

Patients were stratified in two groups according to the percentage variation of SOFA score from T1 to T2 ( $\Delta$ SOFA  $>$  or  $< 20\%$ ).

Because of the small sample size, a dimensionality reduction approach involving minimal-redundancy-maximal-relevance (mRMR) and successively the Elastic Net regression was applied to metabolites concentrations using  $\Delta$ SOFA changes for the binary classification.

Early changes (at T1) in the plasma levels of phosphatidylcholines, lysophosphatidylcholines, sulfoxide-methionine and serotonin were associated with an improvement in organ functions in our model (accuracy from 3 fold cross-validation:  $0.92 \pm 0.08$ ). Moreover, in the course of septic shock, we found a significant different trend in the serotonin/kynurenine ratio between patients with and without improved  $\Delta$ SOFA (unpaired t-test  $p < 0.05$ ). Overall, these findings might indicate novel important pathophysiologic mechanisms with potential implications for early intervention.

**Poster #: 413**

**Abstract #: 2232**

**Abstract Title:** Metabolite profiling of Type-1 diabetes, latent autoimmune diabetes in adults (LADA) and Type-2 diabetes - A metabolic continuum

**Authors:** Mahmoud Al-Majdoub, Arslan Ali, Petter Storm, Anders Rosengren, Leif Groop, Peter Spégel,

**Presenting Author Affiliation:** Lund University Diabetes Centre, Department of Cli

**Abstract Submission:**

Latent autoimmune diabetes in adults (LADA), also called type-1.5 diabetes, is clinically defined as diabetes occurring at <35 years of age with insulin treatment within one year after diagnosis. Due to co-occurrence of the metabolic syndrome, obesity in particular, LADA can be misdiagnosed as type-2 diabetes (T2D).  $\beta$ -cell dysfunction develops much faster in LADA compared to T2D. Hence, accurate diagnosis of LADA is important.

In this study, we examined the circulating metabolome in T2D and LADA to investigate whether levels of metabolites have the ability to differentiate between these two diabetes types. Plasma samples were taken close to diabetes diagnosis from patients with T2D (n=50) and LADA (n=50), using type-1 diabetes (T1D n=50) as a control. Two complementary mass spectrometry-based metabolomics platforms were applied to yield a wide coverage of the metabolome. In total 123 metabolites were identified and relatively quantified.

No unique metabolite marker capable of distinguishing LADA from T2D could be found among the 123 identified metabolites. This notwithstanding, levels of 101 metabolites differed between diabetes types (ANOVA,  $q < 0.05$ ). Instead of showing unique features for the different diabetes types, the metabolome varied along a continuum, extending from T1D via LADA to T2D. Hence, the metabolite profile was largely determined by C-peptide levels. Overall, LADA was more similar to T2D than to T1D. LADA patients showing a metabolite profile that was more similar to T1D progressed faster to insulin therapy than those showing similarities with T2D.

In conclusion, we could not identify any metabolite with the capability to unambiguously distinguish between LADA and T2D. Rather LADA expressed itself as an intermediate of T1D and T2D, with those being more similar to T1D showing a faster progression to insulin treatment than those being more similar to T2D.

**Poster #: 414**

**Abstract #: 2245**

**Abstract Title:** Research into Lysosomal Storage Metabolism using plasma lipid characterization by LC-MS/MS

**Authors:** Sibylle Heidelberger, Daniel Blake, Rachel Webster, Karen Smith, Martin Roch,

**Presenting Author Affiliation:** Sciex

**Abstract Submission:**

Research into plasma sphingolipids and determining the concentrations of such is of growing importance in the clinical research laboratory, particularly within groups researching Lysosomal Storage Metabolism. Current methods of analysis involve either enzyme activity procedures or derivatization of compounds prior to analysis. Direct analysis of these groups can be complex due to extensive structural homogeneity between individual compounds. We present here a method for a direct multi compound screen approach to this analysis, employing modern advances in column technology to produce a rapid and sensitive LC-MS/MS method for these compounds.

Plasma Sphingolipids (Glu-Sph, Gal-Sph, Lyso-Gb3, SPC and the internal standard C-17-SPC) were extracted by solid phase extraction using a method modified (to allow inclusion of additional compounds) from Welford et al (1). Chromatography was performed using a short HILIC column to allow separation of isomeric and isobaric compounds within the group of lipids under investigation. Mass Spectrometry analysis was provided by a SCIEX Triple Quad 6500 LC-MS/MS system operating in Low Mass Positive Turbolonspray mode.

Separation of the structurally similar compounds Glu- and Gal-Sph was achieved. Limits of detection for all compounds was shown to be significantly less than 1ng/ml. Linearity for all compounds investigated was shown to cover at least three orders of magnitude.

We have developed a quantitative method for the extraction and direct analysis of Gal-Sph, Glu-Sph, SPC, Lyso-Gb3 and C17-SPC (internal standard). The method is sensitive and robust with good linearity, and represents a significant improvement on established research methods for investigations of lysosomal storage metabolism. The end result of our proposed method is to provide a significantly enhanced suite of information from a single injection for research into lysosomal storage metabolism.

**Poster #: 415**

**Abstract #: 2272**

**Abstract Title:** Potent inflammatory suppression of the host by the gastro-intestinal nematode, *Haemonchus contortus*.

**Authors:** Gene Wijffels,

**Presenting Author Affiliation:** CSIRO

**Abstract Submission:**

The metabolic effects of parasitism are still being unravelled and thus far metabolomic studies have shown responses that reflect the diversity of parasites and their ecosystems. In this case, infection with the aggressive blood feeding nematode, *H. contortus*, induces a strong suppression of inflammatory signalling in a 20-generation selection line of Susceptible sheep. The Resistance line does not “see” the parasite metabolically.

Alongside several other approaches, we have examined the plasma collected over a time course of primary infection in these Susceptible and Resistant lines of sheep. GC and LCMS of these plasma show that at day 28 of infection in Susceptible line coincides with marked reduction in lipids involved in pro-inflammatory signalling, including EPA, TriHOMEs and prostaglandins. Numerous plasma phospholipids are reduced. The endocannabinoids and tryptophan metabolism is suppressed also indicating altered neurological signalling. There is major disturbance of the TCA cycle and extension to the glycerol-3-phosphate shuttle, and C metabolism.

This metabolic perturbation in the Susceptible line occurs when the nematode is at the adult egg producing stage, feeding voraciously on blood released as it rasps away at the wall of the abomasum (stomach). Both the Resistant and Susceptible lines experience blood loss and consequent drops in RBC count and blood haemoglobin. However, while the Resistant line mounts a florid and persistent eosinophilia, this is completely absent in the susceptible line.

Despite decades of research into the immunology and genetics of susceptibility or resistance to this nematode which causes devastating mortalities and severe morbidity in most sheep and goat production systems, there is still much to learn to curtail the levels of infection and damage from this parasite.

**Poster #: 416**

**Abstract #: 2303**

**Abstract Title:** A GC-MS-based longitudinal untargeted metabolomic analysis of plasma from the Lewis Polycystic Kidney rat model of nephronophthisis

**Authors:** Hayley Abbiss, Garth Maker, Gabrielle Musk, Catherine Rawlinson, Joel Gummer, Trish Fleming, Jacqueline Phillips, Mary Boyce, John Moncur, Robert Trengove,

**Presenting Author Affiliation:** Murdoch University

**Abstract Submission:**

Nephronophthisis (NPHP) is inherited and typically presents with cysts in the kidney and liver, leading to end-stage kidney disease. Detection of NPHP and other polycystic kidney diseases (PKDs) is not sensitive or specific and management and treatment are limited to renal replacement therapy and transplantation. The aim of this study was to use an untargeted metabolomics approach to identify early biomarkers in the blood plasma of the Lewis Polycystic Kidney (LPK) rat model of NPHP. Eleven LPK and 11 Lewis age- and sex-matched control animals aged 5 to 16 weeks were used. Blood was sampled once weekly from the lateral tail vein, cell separated and plasma stored at -80°C. Metabolites were extracted with methanol and water containing 13C6-sorbitol (IS) and were dried in a rotary vacuum concentrator and then frozen. Metabolite extracts were derivatised with methoxyamine-HCl and MSTFA. For the analysis, a Shimadzu QP2010 Ultra GC-MS was used and for data analysis, AnalyzerPro, The Unscrambler X, and SPSS were used. Compounds were matched to an in-house library of metabolites and the NIST mass spectral database. Preliminary data analysis revealed an age effect ( $P < 0.05$ ) for myo-inositol, threonine, lysine, proline, isoleucine, valine, glutamic acid, tyrosine, linoleic acid, arachidonic acid, cholesterol and 1,5-anhydro-D-sorbitol. A strain effect ( $P < 0.05$ ) was found for pyroglutamic acid, citric acid, myo-inositol, serine, glycine, lysine, methionine, leucine, isoleucine, tyrosine, linoleic acid, ornithine, cholesterol, phosphoric acid and 1,5-anhydro-D-sorbitol. These findings are consistent with a previously conducted study utilising kidney and liver tissue where citric acid, serine, tyrosine and phosphoric acid were significantly different between LPK and Lewis kidney tissue and myo-inositol, glycine, cholesterol and phosphoric acid were significantly different between LPK and Lewis liver tissue.



**Poster #: 417**

**Abstract #: 2312**

**Abstract Title:** Lipidomic profiling reveals a novel role of autophagy in myelin lipid breakdown

**Authors:** Marta Iruarizaga-Lejarreta, Jose A. Gomez-Sanchez, Lucy Carty, Enara Arretxe, Cristina Alonso, Rhona Mirsky, Kristján Jessen, Ashwin Woodhoo,

**Presenting Author Affiliation:** One Way Liver S.L.

**Abstract Submission:**

Although Schwann cell myelin breakdown is the universal outcome of a remarkably wide range of conditions that cause disease or injury to peripheral nerves, the cellular and molecular mechanisms that make Schwann cell-mediated myelin digestion possible have not been established.

Here we evaluate the possible role of autophagy in mediating myelin degradation in response to nerve injury. For this purpose, we examined the grade of myelin lipid degradation in WT and Atg7 cKO mice, which shows an impaired autophagy specifically in Schwann cells.

Lipidomic profiling of purified myelin fractions, which reflects the grade of myelin degradation, from control and cut nerves obtained from both WT and Atg7 cKO mice was performed. A specific UPLC-time-of-flight-MS based platform analyzing chloroform/methanol extracts was used for optimal profiling of the different lipid species, including fatty acyls, glycerophospholipids, glycerolipids, sphingolipids and sterol lipids. Approximately, 300 metabolites were determined.

Lipidomic profiling showed that the typical down-regulation or up-regulation of a large proportion of lipid species seen in WT myelin was notably impaired in Atg7 cKO myelin. Furthermore, up-regulation of cholesteryl esters, which are by-products of myelin lipid breakdown, was markedly lower in Atg7 cKO nerves than in WT ones, indicating a slower myelin breakdown.

Overall, these lipidomic results demonstrate that genetic inhibition of autophagy results in aberrant breakdown of the lipid component of the myelin sheath, suggesting the role of autophagy in mediating the degradation of myelin after nerve injury.

**Poster #: 418**

**Abstract #: 2321**

**Abstract Title:** GC-MS-based metabolomic analysis indicates persistent differences in the plasma of patients suffering of hyperthyroidism also after treatment.

**Authors:** Simone Poddighe, Sonia Liggi, Cristina Piras, Nicolò Arisci, Giacomo Marini, Stefano Mariotti, Luigi Atzori,

**Presenting Author Affiliation:** Department of Biomedical Sciences - University of

**Abstract Submission:**

The imbalance of thyroid hormones leads to endocrine disorder and it is strongly correlated with major co-morbidity and high risk of mortality. Hyperthyroidism is a common pathological condition characterized by high thyroid hormone (T3, T4) and low serum TSH concentration. To date, there is a lack of metabolomic investigations on patients suffering of hyperthyroidism (HThy) and on the effects of hormonal therapy on their metabolome. The aim of the study was to define and discriminate the metabolic profile of a group of HThys from a panel of healthy euthyroid (HC) with a GC-MS-based metabolomic approach using blood plasma before and after methimazole (MMI) treatment. Samples were collected from HThys at the moment of diagnosis (t0) and when after 3-4 months of MMI therapy, when the levels of circulating free T3 (FT3), free T4 (FT4) and TSH were in the physiological range (t1). Data analysis was conducted with principal components analysis followed by a supervised analysis (PLS-DA), yielding predictive differences in the metabolome at t0 between HThys and HCs (R2X 0.317 R2Y 0.809 Q2 0.583  $p < 0.001$ ).

Sixteen discriminant metabolites were identified and used to detect the major pathways that were significantly altered and impacted via MetaboAnalyst 3.0. The pathways analysis indicates aminoacyl-tRNA biosynthesis, lysine degradation, arginine and proline, glycine, serine and threonine metabolisms as the more affected pathways. Similar results were obtained when the comparison between HThys to HCs was at t1, indicating that pathological alterations are still present in HThys after a short period of therapy.

Our results suggest persistent metabolic alterations in spite of “normal” physiological values of thyroid hormone and might indicate more sensitive biomarkers for patients with hyperthyroidism. Furthermore, our approach confirm metabolomics as a valid non-invasive methods for the study of endocrine disorders. Additional studies on the long-term effects of hormonal therapy are in progress.

**Poster #: 419**

**Abstract #: 2395**

**Abstract Title:** Discrimination of cancerous and non-cancerous lung cell lines by SIFT-MS and GC-QTOF/MS

**Authors:** ZHUNAN JIA,

**Presenting Author Affiliation:** National University of Singapore Graduate School f

**Abstract Submission:**

Selected Ion Flow Tube Mass Spectrometry (SIFT-MS) and Gas Chromatography Mass Spectrometer (GC/MS) has been used to analyze the volatile organic compounds (VOCs) in the headspace of lung cell line spent medium. The two cell lines selected were MRC-5 (human healthy lung fibroblast cells) and NCI-H460 (large cell lung cancer cell derived from pleural effusion site).  $1 \times 10^5$  of cells were seeded, after 72 hours growth at 37°C in 5% CO<sub>2</sub>, the spent medium were collected and separated into two bathes for concurrent run with SIFT-MS and GC-MS. For SIFT-MS, the headspace VOCs were directly feed into the instrument by puncturing the sample inlet needle into the bottle septum, for GC-MS, solid phase micro extraction was used. 12 replicates were analyzed for each cell line. VOCs from pure medium were analyzed in the same way to serve as control. Principle component analysis of the result demonstrates that there is clear distinction in VOCs profiles from non-cancerous cell, cancerous cell and pure medium. Among the discriminating compounds identified based on Variable Importance Projection (VIP) scores and P values from data obtained by both techniques, styrene was found to be increased significantly in healthy cell medium headspace and decreased significantly in the case of cancer cell. Benzene and 1, 3-bis (1, 1-dimethylethyl)-benzene were also found to be increased in the headspace of healthy lung cell. 2-ethyl-1-hexanol was found to be emitted by lung cancer cell. To the best of our knowledge, this is the first study of VOCs from MRC-5 and NCI-H460 cell lines. We conclude that this lung cancer cell line can release a characteristic odor whose constituents may be used as volatile disease markers.

**Poster #: 420**

**Abstract #: 2406**

**Abstract Title:** In silico identification of a novel transcription factor associated with carotenoid biosynthesis in Zea mays

**Authors:** Zahra Zinati, Ali Behpouri,

**Presenting Author Affiliation:** Department of Agroecology, Darab College of Agriculture

**Abstract Submission:**

In silico identification of a novel transcription factor associated with carotenoid biosynthesis in Zea mays

Zahra Zinati and Ali Behpouri

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**Abstract**

Phytoene synthase (PSY), which is encoded by the phytoene synthase 1 (PSY1) gene, is the first rate-limiting enzyme in the plant carotenoid biosynthetic pathway. PSY1 (accession: GRMZM2G300348) gene is related to endosperm color and carotenoid accumulation in maize kernels. Many researches show the prevention and treatment properties of carotenoids against several kinds of diseases, such as certain chronic disease, cardiovascular disease as well as certain cancers. These interesting properties of carotenoid have attracted intensive works in carotenoid biosynthesis. Computational analysis makes it more feasible to unfold regulatory networks underlying the carotenoid biosynthesis. In doing so, some of bioinformatics servers and databases such as Phytozome, PlantPAN (Plant Promoter Analysis Navigator) and PlantTFDB (Plant Transcription Factor Database) were used to analyze PSY1 gene in more detail and identify coexpressed transcription factors which bind to the PSY promoter region. The most significant result revealed from this study can be presented in the discovery of one transcription factor (accession: GRMZM2G130442 Homeodomain leucine zipper family IV protein Outer cell layer5a Uncharacterized protein) belonging to HB family that is coexpressed with four identified transcripts (GRMZM2G300348\_T01, GRMZM2G300348\_T02, GRMZM2G300348\_T03, GRMZM2G300348\_T04) of PSY1 gene under various conditions (environmental stresses and hormone treatments.) It is therefore likely that this transcription factor may act as critical regulator of PSY1 gene expression. Identification of the protein acting upstream of PSY1 within the Zea mays will shed light on the fine tuning of PSY genes. Such understanding would also contribute to metabolic engineering of enhanced carotenoid biosynthesis.

**Keywords:** Phytoene synthase, In silico, Zea mays, transcription factor

**Poster #: 421**

**Abstract #: 2443**

**Abstract Title:** Metabolic variation in cultured cells treated with differentially functionalised gold nanoparticles (GNPs).

**Authors:** Jeremie Lindeque,

**Presenting Author Affiliation:** Human Metabolomics, North-West University (Potchef

**Abstract Submission:**

Recently, there have been a strong movement towards the development of nano-medication and other nano-based pharmaceutical applications. Gold nanoparticles (GNPs) in particular have shown beneficial outcomes in targeted drug delivery, treatment of various diseases (including cancer) and colorimetric diagnostics. In addition, research on nano-material safety has had increased amount of attention as cytotoxicity in certain studies have been reported. While the effect of these particles has been studied on several biochemical levels, the effect GNPs have on the metabolome of living organisms is commonly overlooked, not to mention the effect various coated or functionalised GNPs has. With this study we aimed to elucidate the effect of different functionalised GNPs on the endo- and exometabolome of cell cultures using targeted and untargeted metabolomic methods. HepG2 cells were cultured and treated with different coated GNPs. Mitochondrial metabolism (respiration) was measured using the Seahorse. The endometabolome of the GNP treated cells were extracted and analysed on GC-MS and LC-MS/MS. The culture media were deproteinated and analysed with NMR. Comparison of the GNP treated cells with control cells showed altered mitochondrial respiration and variations in the levels of several metabolites. The results indicate that the so-called bio-inert gold particles do affect the metabolism of cells and could explain the cytotoxicity seen in several studies. While research on different cells and higher organisms is needed, it is recommended that this effect be kept in mind when designing nano-medicine.

**Poster #: 422**

**Abstract #: 2492**

**Abstract Title:** Metabolomics as a tool for the study of drug response in multiple sclerosis

**Authors:** federica murgia, eleonora cocco, lorena loreface, simone poddighe, maria rita murru, maria giovanna marrosu, luigi atzori,

**Presenting Author Affiliation:** University of Cagliari

**Abstract Submission:**

Multiple sclerosis (MS) is a chronic disease of the central nervous system characterized by high levels of heterogeneity in pathological, clinical, and radiological features, as well as drug responses. It is characterized by the formation of sclerotic plaques in various areas of the central nervous system. These plaques are the result of an inflammatory response most likely caused by activation of autoimmune Th-1 cell targeting oligodendrocytes and the myelin sheath together with activated monocytic cells. Different kinds of therapy have been proposed. Interferon  $\beta$  is a glycoprotein endowed with immunomodulatory properties. The exact mechanism of action of Interferon  $\beta$  in multiple sclerosis is still under investigation. Metabolomics is a non-selective approach with the potential to discover new biomarkers for the study of the mechanism of action of drugs, and is crucial for to investigate metabolic changes during treatment in patients. In the present study, plasma samples from 16 MS patients under treatment with Interferon  $\beta$  were collected at four different time point (T0 (without treatment), 0, 12months) and analyzed by  $^1\text{H}$ -NMR spectroscopy. Data analysis was conducted with principal components analysis followed by a supervised analysis (OPLS-DA). The metabolites were identified and quantified and metabolic pathways characterization performed. OPLS-DA models were built comparing the T0 with the consecutive time points. The most discriminant model was T0 versus T>12. The metabolic trends indicated a decrease of ketone bodies (high in T0) during the treatment and an increase of tryptophan (lower in T0). In conclusion, metabolomic analysis was able to discriminate different metabolic profiles in MS patients during the treatment with Interferon  $\beta$ . The main metabolic changes could be connected to two different metabolic pathways: tryptophan/kynurenine and energy metabolism. So, metabolomics represents a promising non-invasive approach for the study of the drug response in MS.

**Poster #: 423**

**Abstract #: 2528**

**Abstract Title:** Influence of mass resolving power in high resolution mass spectrometry metabolomics

**Authors:** Lukáš Najdekr, David Friedecký, Tomáš Pluskal, Junhua Wang, Ralf Tautenhahn, Yingying Huang, Tomáš Adam,

**Presenting Author Affiliation:** Institute of Molecular and Translational Medicine,

**Abstract Submission:**

Modern separation methods in conjunction with high resolution accurate mass (HRAM) spectrometry can provide an enormous number of features characterized by exact mass and by chromatographic behavior. Higher mass resolving power requires usually longer scanning times and thus less data points across the peak are acquired. This could present an issue in quantification, component detection and possible problem with deconvolution. The aim of this work is to describe influence of mass spectrometry resolving power on profiling metabolomics experiments.

From metabolic databases (HMDB, LipidMaps, KEGG) a list of compounds (41 474) was compiled and potential adducts, isotopes were calculated (622 110 components). Number of distinguishable masses was calculated for up to 3840k resolution. To evaluate these models, LC-HRMS of single plasma sample was run on Orbitrap Elite (Thermo Fisher Scientific, CA, USA) at resolving power settings 15k up to maximum 480k. For evaluation software XCMS 1.44, MZmine 2.13.1 and Sieve 2.2SP2 were used. Distribution graphs based on m/z values were made in order to determine which intervals of m/z are influenced the most.

In plasma samples number of detected components by three software was peaking at 120k (Sieve (18268), MZmine (8861), XCMS (5538)) in positive mode and at 60k (Sieve (1008), MZmine (1879), XCMS (1149)) in negative mode. With increasing mass spectrometry resolving power the number of components was decreasing in both ionization modes. One of the reasons of this behavior could be lower number of data-points acquired through analyses.

In conclusion the most effective mass resolving power for profiling analyses of metabolite rich biofluids on Orbitrap Elite are 120k in positive mode and 60k in negative mode. Region between 400 – 800 m/z is influenced by resolution the most.

**Poster #: 424**

**Abstract #: 2547**

**Abstract Title:** PhenoMeNal: Large Scale Computing for Medical Phenotyping Data

**Authors:** Pablo Moreno,

**Presenting Author Affiliation:** EMBL-EBI

**Abstract Submission:**

The amount of data generated by molecular phenotyping exceeds the data volume of personal genomes by at least an order of magnitude. The collection of such information will pose dramatic demands on biomedical data management and compute capabilities. For example, a single typical National Phenome Centre in the UK, managing only around 100,000 human samples per year, can generate more than two petabytes of data yearly.

The PhenoMeNal Project will develop and deploy an integrated, secure, permanent, on-demand service-driven, privacy-compliant and sustainable e-infrastructure for the processing, analysis and mining of the massive amount of molecular phenotyping and genotyping data that will be generated by metabolomics applications now entering research and clinic.

This e-infrastructure will support the data processing and analysis pipelines for molecular phenotype data from the earliest time point of data acquisition in the laboratory up to the high level medical and biological conclusions and interpretations. This will allow small and large laboratories, with access ranging from cloud infrastructure and HPC to no computing power at all, to process their large and small scale data sets with workflows that use state-of-the-art software tools, either on public clouds, private clouds or hardware set at their own institutions.

After 9 months of started the project, we have conducted UX sessions, staff exchanges, outreach activities, regular online meetings and three technical workshops. Here we report our current progress in e-infrastructure setup and deployment, tools packaged, data federation resolutions, workflow environment integration/tests, outreach and main use cases chosen. We aim to interact with the attendants of the Metabolomics Society 2016 conference to further improve our understanding of their metabolomics data handling and analysis needs.



**Poster #: 425**

**Abstract #: 2559**

**Abstract Title:** Red blood cells' metabolome evaluation in blood bank conditions

**Authors:** Thelma Pertinhez,

**Presenting Author Affiliation:** University of Parma

**Abstract Submission:**

Blood transfusion is a fundamental therapy in numerous pathological conditions. Many studies in the past years have been aimed to the identification of biomarkers of storage lesions and to the evaluation of the quality of red blood cells (RBCs) conserved in blood banks. Among the storage processes routinely performed on RBCs, we choose to study a) leukodepletion, a pre-storage treatment recognized to better preserve the quality of RBCs overtime, and b) gamma-irradiation employed to reduce the risk of graft-versus-host disease (GvHD) in immune-compromised patients.

Erythrocytes concentrates from donors were prepared as leukoreduced and pre-storage leukodepleted, re-suspended in SAGM and then analyzed during 6 weeks. In parallel, the effect of different doses of gamma-irradiation on some of leukodepleted units was followed for 2 weeks.

The metabolic profiles were evaluated by <sup>1</sup>H-NMR spectroscopy data have been combined with clinical laboratory assays, such as degree of hemolysis, electrolytes and ammonium concentration.

Overall the data indicate that leukodepletion provides RBCs with an appropriate amount of nutrients for longer time, selects RBCs less prone to storage lesions and prolongs their viability.

We observed variation of amino acids metabolism in a time- and gamma-irradiation-dose dependent way: in particular an increment of ammonium, potentially toxic for critical patients (massive transfusion and bone marrow transplant).

The obtained analytical data offer new solid hints to further optimize the current storage protocols.

**Poster #: 426**

**Abstract #: 2568**

**Abstract Title:** Pharmacometabolomic Signatures of Ketamine and Esketamine in Subjects with Refractory Major Depressive Disorder

**Authors:** Daniel Rotroff, Wayne Drevets, Giacomo Salvatore, Rima Kaddurah-Daouk,

**Presenting Author Affiliation:** NC State University

**Abstract Submission:**

Background: Ketamine, at sub-anesthetic doses, is reported to rapidly decrease depression symptoms in patients with treatment-resistant major depressive disorder (MDD). Many patients do not respond to currently available antidepressants, making ketamine and its S-enantiomer, esketamine, an attractive option for treatment resistant MDD. Nevertheless, the mechanism(s) by which ketamine/esketamine may produce antidepressant effects are not well understood. Here we use a pharmacometabolomics approach to map acute global metabolic effects of these drugs in treatment-refractory MDD patients.

Methods: Plasma was collected upon 2 hours from infusion with ketamine (n=33) or esketamine (n=20). Two complementary metabolomics platforms were used providing broad biochemical coverage of 504 known and unknown metabolites. Effects of esketamine on metabolism were tested again in the same esketamine subjects by repeating exposure 4 days later. In addition, changes in metabolites were correlated with changes in depression severity as assessed by the Montgomery–Åsberg Depression Rating Scale. All tests were conducted using non-parametric, Wilcoxon signed-rank tests and adjusted using a false discovery rate approach ( $q < 0.25$ ).

Results: Both drugs significantly and rapidly altered metabolites related to tryptophan metabolism and the urea cycle ( $q < 0.25$ ). We obtained evidence of the known downstream effects of NMDA antagonism and observed changes in glutamate. Also, we identified impacts on circulating phospholipids that associate with clinical improvements.

Conclusions: Overall, these data present new insights and further elucidate the underlying mechanism of ketamine and esketamine drug effects and represent some of the first detailed metabolomics mapping for these promising therapies.

**Poster #: 427**

**Abstract #: 2577**

**Abstract Title:** Metabolomic analyses of the Cerebrospinal Fluid in Fibromyalgia

**Authors:** Payam Emami Khoonsari, Stephanie Herman, Obaid Aftab, Mats Gustafsson, Ola Spjuth, Lars Tanum, Torsten Gordh, Kim Kultima,

**Presenting Author Affiliation:** Dept. of Medical Sci., CARAMBA, Uppsala University

**Abstract Submission:**

Introduction

Fibromyalgia is a chronic pain condition that affects between three and six percent of the worldwide population (primarily women of childbearing age). The disorder is characterized by widespread pain including symptoms such as fatigue, muscle stiffness, and somnopathy. Some of the symptoms are also found in other disorders, making fibromyalgia difficult to diagnose before ruling out other conditions. The aim of this study was to examine whether fibromyalgia patients have elevated cortisol levels compared to controls and to detect novel metabolites in cerebrospinal fluid that can be used to diagnose fibromyalgia.

Methods

Internal standards in chilled methanol were added to crude CSF followed by vortexing, centrifugation and drying using centrifugal vacuum. The precipitate was dissolved in methanol, separated using an HPLC system and injected into a Q Exactive™ Hybrid Quadrupole-Orbitrap (Thermo Scientific). Metabolomic data was acquired using positive and negative polarity modes. The preprocessing and quantification was performed using OpenMS followed by identification using Human Metabolome Database and The METLIN Metabolomics Database, in OpenMS and XCMS respectively.

Results

Our results showed no indication of elevated levels of cortisol in fibromyalgia patients compared to controls ( $p=0.29$ ). Using an untargeted metabolomic approach, we identified 30 metabolites with altered levels in patients suffering from fibromyalgia compared to controls. Two of these metabolites, N-Acetyl aspartyl glutamic acid ( $p=0.007$ ) and L-Serine ( $p=0.006$ ), have previously been found to be altered in patients suffering from fibromyalgia. The remaining metabolites have previously not been found in association with fibromyalgia and therefore represent novel finding in association with the disease with a potential use in diagnosing fibromyalgia.

**Poster #: 428**

**Abstract #: 2323**

**Abstract Title:** Antibiotic-induced shifts in gut microbial metabolomes

**Authors:** Alesia Walker, Monika Schaubeck, Thomas Clavel, Dirk Haller, Philippe Schmitt-Kopplin,

**Presenting Author Affiliation:** Helmholtz Zentrum München Deutsches Forschungszentrum

**Abstract Submission:**

The aim of the present study was to investigate the effect of antibiotics on gut microbial metabolism in C57BL/6N mice using non-targeted metabolomics. Cecal contents were extracted with cold methanol and metabolites were analyzed by high-resolution mass spectrometry (MS), including both direct infusion FT-ICR-MS and reversed-phase chromatography with subsequent identification by means of UPLC-MS/MS to enlarge metabolite coverage. Antibiotic treatments induced marked changes in intestinal bacterial communities and had also a major impact on cecal metabolome, affecting different metabolite classes such as secondary bile acids, short chain fatty acids, lipids and sulfated flavonoids. A correlation analysis was applied to reveal microbiome-metabolome connections and visualized by a force-based layout algorithm (AllegroLayout). This showed for example that the family Lactobacillaceae and the metabolite lactic acid had an important role in microbiome-metabolome interactions. A similar approach will be used to identify unknown metabolites, which is a key challenge in metabolomics research. In summary, antibiotics drive dramatic changes in gut microbiome and can facilitate the identification of gut microbial metabolites.

**Poster #: 429**

**Abstract #: 2015**

**Abstract Title:** Host Cellular Metabolic Responses and Potential Pathogenesis of Chronic Hepatitis B Virus Infection

**Authors:** Hongde Li, Xinwen Chen, Lin Guo, Huiru Tang, Yulan Wang,

**Presenting Author Affiliation:**

**Abstract Submission:**

Background

Infection caused by the chronic hepatitis B virus (HBV), is strongly associated with hepatitis, fatty liver and hepatocellular carcinoma. The underlying mechanisms of HBV-induced diseases, however, are not completely understood, and there are currently no cures for this chronic infection. As viruses depend on host cellular metabolism to reproduce, we hypothesize that investigating the host-virus interactions, focusing mainly on metabolism, could provide some clues to the mechanism of infection and some important information on drug targets for treating HBV infection. Here, we used metabonomics and molecular biological assays to characterize the metabolic features of host cells.

Results

We show that HBV replication induces systematic metabolic alterations in host cells. HBV replication induces the promotions of central carbon metabolism, biosynthesis of nucleotides and total fatty acids. HBV replication also up-regulates the biosynthesis of hexosamine and phosphatidylcholine by activating glutamine-fructose-6-phosphate amidotransferase 1 (GFAT1) and choline kinase alpha (CHKA) respectively. Furthermore, we demonstrate that suppressing hexosamine biosynthesis and phosphatidylcholine biosynthesis can inhibit HBV replication and expression, suggesting that GFAT1 and CHKA could be potential antiviral drug targets. Importantly, up-regulation of GFAT1 and CHKA are known to contribute to carcinogenesis. HBV-induced fatty liver and hepatitis may be attributed, at least in part, to GFAT1 activated hexosamine biosynthesis.

Conclusions

The present study used metabolic profile analysis to reveal the host-HBV interactions. HBV can modulate the host metabolic program to benefit its replication, while the metabolic alterations could contribute to disease. Taken together, these observations provide further insight into the pathogenesis of HBV-induced diseases, and shed new light on drug targets for treating HBV infection.

**Poster #: 430**

**Abstract #: 2050**

**Abstract Title:** Metabolic changes in serum and urine during pregnancy with gestational diabetes mellitus

**Authors:** Guiping Shen,

**Presenting Author Affiliation:**

**Abstract Submission:**

**Introduction:** Gestational Diabetes Mellitus (GDM) is one of the most challenging health problems during and after pregnancy, and its diagnosis in early pregnancy is quite difficult but to be vitally necessary. In this study, metabolic changes in serum and urine during pregnancy were described to identify novel biomarkers for the early prenatal diagnostics and prognostics of GDM.

**Methods:** In the present study, serum and urine samples were collected from GDM and healthy pregnant women at the age of 25 ~ 34 years and 28 ~ 32 gestational weeks. After the preprocessing of these samples, <sup>1</sup>H nuclear magnetic resonance (NMR) spectroscopy combined with multivariate statistical analysis was applied to identify the potential metabolic signatures of GDM and their corresponding metabolic pathways.

**Results:** In our result, an untargeted metabolomics analysis revealed some characteristic metabolites changes including glucose, amino acids, lipids and proteins between GDM and healthy pregnancy women in serum and urine. Compared with the healthy pregnancy, trimethylamino oxide (TMAO), glycine and glucose were found to increase significantly in serum of GDM, and the urine demonstrated some similar variation trends in glucose, acetoacetate, lactate and 3-hydroxybutyrate in GDM, however, malonate, hippurate, ethanolamine, glutamate and phenylacetylglycine (PAG) demonstrated different degrees of reduction. These variations in serum and urine indicate the specific metabolites to distinguish GDM from healthy pregnancy, which could serve to the earlier diagnosis of GDM.

**Conclusion:** Our study show that NMR-based metabolomics could be a promising approach in physiopathologic metabolism investigation and early diagnosis of GDM.

**Poster #: 431**

**Abstract #: 2143**

**Abstract Title:** HYPERGLYCEMIA-RELATED ADVANCED GLYCATION END-PRODUCTS IS ASSOCIATED WITH THE ALTERED PHOSPHATIDYLCHOLINE METABOLISM IN OSTEOARTHRITIS PATIENTS

**Authors:** weidong zhang, guang sun, Sergei Likhodii, Ed Randell, Andrew Furey, guangju zhai,

**Presenting Author Affiliation:** memorial university of newfoundland

**Abstract Submission:**

**Objectives:** We previously found that altered phosphatidylcholine metabolism is responsible for the association between osteoarthritis (OA) and diabetes mellitus (DM) and hypothesized hyperglycemia-related production of advanced glycation end-products (AGEs) was involved in the altered phosphatidylcholine metabolism in OA patients and tested the hypothesis in the current study.

**Methods:** Synovial fluid and plasma samples were collected from OA patients with and without DM. Hyperglycemia-related AGEs including methylglyoxal (MG) and methylglyoxal-derived hydroimidazolone (MG-H1) levels were measured using UPLC/MS method. The correlation between MG, MG-H1, and phosphatidylcholine acyl-alkyl C34:3 (PC ae C34:3) and phosphatidylcholine acyl-alkyl C36:3 (PC ae C36:3) were examined.

**Results:** 84 knee OA patients, including 46 with DM and 38 without DM, were included in the study. We did not find a

significant difference in plasma MG-H1 concentration between OA with and without DM. However, we found that synovial concentrations of MG-H1 in the groups of OA with DM were  $2.56 \pm 0.27$  ng/ml which was significantly higher than that in the group of OA without DM ( $2.39 \pm 0.25$  ng/ml,  $P=0.012$ ). Similarly, synovial concentration of MG was  $2.05 \pm 0.11$  ng/ml in the group of OA with DM which was significantly higher than  $1.99 \pm 0.11$  ng/ml in the group of OA without DM ( $P=0.046$ ). The significance remained after adjusting the age, BMI and sex. The correlation between MG-H1 and PC ae C34:3, MG-H1 and PC ae C36:3, MG and PC ae C34:3, and MG and PC ae C36:3 were -0.15, -0.32, -0.23 and -0.06, respectively.

**Conclusions:** We demonstrated that both MG-H1 and MG concentrations in synovial fluid were elevated in OA patients

with DM and associated with the levels of PC ae C34:3 and PC ae C36:3, suggesting that hyperglycemia-related AGEs

may be responsible for the altered phosphatidylcholine metabolism in OA.

**Poster #: 432****Abstract #: 2191**

**Abstract Title:** The impact of short-term exposure to disinfection by-products on the metabolome – a metabolome-wide association study

**Authors:** Karin van Veldhoven, Dinesh Barupal, Pekka Keski-Rahkonen<sup>2</sup>, Lutzen Portengen, Laia Font-Ribera, Cristina M Villanueva, Florence Guida, Paolo Vineis, Augustin Scalbert, Roel Vermeulen, Manolis Kogevinas, Marc Chadeau-Hyam,

**Presenting Author Affiliation:** Imperial College London

**Abstract Submission:**

## Introduction

Exposure to disinfection by-products (DBPs) through drinking water and chlorinated swimming pools has been associated with adverse health outcomes such as bladder cancer and impaired respiratory health. The underlying biological mechanisms remain unknown and therefore the aim of this study was to investigate the impact of DBPs on the metabolome.

## Methods

We used data from the PISCINA study, part of the EU-funded EXPOSOMICS project. This short-term experimental study was performed in an indoor chlorinated pool where 60 volunteers (18-40y non-smokers) swam for 40 minutes. Questionnaires about lifestyle and physical activity were completed, heart rate was monitored, and exhaled breath and blood samples were collected before and 2 hours after swimming. Exposure to DBPs was assessed using measurements of chloroform, bromodichloromethane, dibromochloromethane, and bromoform in exhaled breath. Untargeted blood-derived metabolomics was conducted using a UHPLC-QTOF mass spectrometer with reversed phase column and electrospray ionization in positive polarity. Features with less than 40% missing were imputed. Associations between DBP-exposure and levels of metabolites were analysed using multivariate normal (MVN) regression models, and series of sensitivity analyses were performed.

## Results

A total of 6,471 metabolic features were included in the analyses. All exposures were significantly higher after the experiment. Preliminary analyses suggested that metabolic profiles from samples before and after swimming can be clearly discriminated. MVN-models showed that 52 metabolomic features were associated with all exposures and that 1, 3, and 6 metabolomic features were only associated with bromoform, the sum of the exposures, and bromodichloromethane, respectively (Bonferroni 5% level).

## Conclusion

All metabolic features are unknown and additional annotation will be needed to provide insights into metabolic changes induced by exposures to DBPs, potentially shedding light on biological pathways affected by these exposures, and in-turn affecting risk of adverse health outcomes.



**Poster #: 433**

**Abstract #: 2356**

**Abstract Title:** Semi-targeted GC-MS method for metabolomics analysis of urinary sugar species

**Authors:** Carina Mack, Christoph Weinert, Björn Egert, Eva Hummel, Achim Bub, Bernhard Watzl, Sabine Kulling,

**Presenting Author Affiliation:** Max Rubner-Institut

**Abstract Submission:**

**Background:** Despite the high complexity of urinary sugar profiles, so far analytical methods developed for the analysis of sugar species in urine usually target only common monosaccharides and polyols. The semi-targeted determination of a broader set of sugar species in urine is difficult for two reasons: Firstly, while mass spectra are often too unspecific to enable separation of the (in part) highly similar isomeric compounds, many stationary phases of GC columns also do not provide sufficient selectivity. Secondly, there is a lack of sensitivity in detecting minor sugar species. A multiplatform approach could solve these problems, but would necessitate at least two analytical runs per sample. This shows the need for a semi-targeted metabolomics method enabling the simultaneous detection of known and unknown sugar species.

**Method:** 24 h urine samples obtained from the KarMeN study (Karlsruhe Metabolomics and Nutrition), a human metabolomics study with healthy participants on an unrestricted diet, were analyzed using a semi-targeted GC-MS method. Urine samples were normalized on osmolarity and evaporated. Thereafter samples were methoximated and trimethylsilylated and then analyzed. A Scan-/SIM-approach allowed the monitoring of known and unknown sugar species using typical mass fragments.

**Results:** Using the semi-targeted GC-MS method, up to 55 different known and unknown sugar species were detected in human urine. Of these, 38 were identified. The Scan-/SIM-approach enabled the separation and relative quantification of major sugar species such as mannitol and minor sugar species like sedoheptulose or maltose. The relative standard deviation of the internal standards measured in 456 study and quality control samples showed a high long-term reproducibility (10.5-13.4 % and 3.6-5.4 % before and after signal intensity drift/batch correction, respectively). Based on this data set, e.g. markers for the consumption of dairy products as well as clear sex-specific differences were found.

**Poster #: 434**

**Abstract #: 2360**

**Abstract Title:** Accumulation of triacylglycerols in Arabidopsis during heat acclimation

**Authors:** Stephanie Mueller,

**Presenting Author Affiliation:** German

**Abstract Submission:**

Plants are exposed to high temperature fluctuations mainly during hot summer days. Temperatures are typically lowest in the morning and reach a maximum in the afternoon. Plants have the inherent capacity to acquire tolerance to otherwise lethal heat temperatures, and, therefore can tolerate and survive short-term heat stress even on hot summer days through heat acclimation. Arabidopsis seedlings acclimate at moderately elevated temperatures between 32 – 38° C. During heat acclimation, a genetically programmed heat shock response (HSR) is triggered that is characterized by rapid activation of heat shock transcription factors (HSF) which trigger a massive accumulation of a battery of heat shock proteins, chiefly involved in protein folding and protection.

However, little is known on the metabolic adjustments during heat acclimation. In order to identify metabolites responding to elevated temperatures, global metabolite profiles of heat-acclimated and control seedlings were compared. Untargeted metabolite analyses revealed that levels of polyunsaturated triacylglycerols (TAG) rapidly increase during heat acclimation. TG accumulation was found to be temperature dependent in a temperature range from 32 – 50° C (optimum at 42° C) and reversible after return from 37° C to normal growth temperatures. Heat-induced TGs accumulated in extrachloroplastic compartments and increased both in roots and shoots to a similar extent. Analysis of mutants deficient in all four HSFA1 master regulator genes or the HSFA2 gene revealed that TG accumulation was not dependent on HSFs. Moreover, the TG response was not limited to heat stress since drought and salt stress also triggered an accumulation of TGs, but not short-term osmotic, cold and high light stress. Lipid analysis revealed that heat-induced accumulation of TGs is not due to massive de novo fatty acid synthesis. It is hypothesized that TGs serve as transient stores for fatty acids that may be required for membrane remodeling during heat acclimation.

**Poster #: 435**

**Abstract #: 2370**

**Abstract Title:** Metabolomic changes during compensatory evolution following gene loss in *Saccharomyces cerevisiae*

**Authors:** Roland Tengölics, Kalapis Dorottya, Balázs Szappanos, Gábor Rákhely, Csaba Pál, Balázs Papp,

**Presenting Author Affiliation:** HAS Biological Research Centre Ins of Biochemistry

**Abstract Submission:**

Deleterious mutations generate a strong selection pressure to recover fitness through compensatory genetic changes. Understanding how metabolic networks are rewired during compensatory evolution has relevance not only for evolutionary biology but also for bioengineering where genetic modifications with deleterious metabolic side effects are common. Despite its central importance, metabolic reprogramming upon gene deletion and following compensatory adaptation remains poorly studied.

To begin to address this gap in our knowledge, here we focus on yeast metabolism as a model system and measure metabolic phenotypes of dozens of strains carrying gene deletions and compensatory mutations generated in a previous study (Szamecz et al. 2014). The fast growth of *Saccharomyces cerevisiae* strongly depends on the overflow metabolite production through cofactor regeneration (Merico et al. 2007), while overflow metabolite production reflects to intracellular flux states (Mo et al. 2009). Therefore monitoring the production rates of overflow metabolites gives valuable phenotypic data to decipher the metabolic changes accompanying compensatory evolution.

We applied a high-throughput overflow metabolite production screen on 23 gene deletant and 54 corresponding laboratory-evolved compensated strains to address the following specific questions: 1) Does the extent of metabolic reprogramming correlate with the degree of compensation (i.e. growth recovery)? 2) By studying strains that show a high degree of compensation, do we find evidence that numerous distinct overflow metabolite production patterns with wild-type like growth rates are accessible through compensatory mutations? 3 By analyzing the overflow metabolite production of parallelly evolved lines (same single gene knockout background), do we see similar overflow metabolite production patterns, or divergence is common between these lines?

**Poster #: 436**

**Abstract #: 2520**

**Abstract Title:** Endogenous metabolites as more sensitive markers of CYP3A4 activity in inhibition and induction state than midazolam clearance

**Authors:** Jieon Lee,

**Presenting Author Affiliation:** Department of Clinical Pharmacology and Therapeuti

**Abstract Submission:**

Cytochrome P450 (CYP) 3A4, the most abundant cytochrome isoform in humans, metabolizes a large number of pharmacological and endogenous substances. Evaluation of individual CYP3A4 activity is a great importance within the drug development and in clinical practice because of increasing of poly-pharmacy. This study aimed to identify and validate endogenous metabolites as markers of CYP3A4 activity in CYP3A4 induction and inhibition states with different extents of induction and inhibition. We conducted two clinical studies A total of 48 healthy male adults (24 subjects in each study) administrated midazolam alone or pretreated with different CYP3A4 inhibitor such as itraconazole 200 mg or ketoconazole 400 mg (inhibition state) and inducers such as rifampicin 150 mg or rifampicin 600 mg (induction state). During each study period, urine samples were collected during 12 hours intervals for metabolic analysis before and after the administration of midazolam. We quantitated the concentration of several endogenous metabolites in urine by gas chromatography-mass spectrometry. Midazolam clearance fold changes relative to baseline levels were not significantly different after administration of midazolam pretreated with different inhibitor or inducer. On the other hand, the extents of 6 $\beta$ -OH-cortisol/cortisol ratios relative to their baseline were 0.51, 0.18-fold after administration of itraconazole 200 mg and ketoconazole 400 mg, respectively, and 3.55, 5.88-fold after administration of rifampicin 150 mg and 600 mg, respectively. Similarly, the extent of their 6 $\beta$ -OH-cortisone/cortisone changes adjusted by baseline levels were significantly different in both inhibition state (0.54, 0.21-fold) and induction state (2.9, 5.1-fold) with different inhibitor and inducer. Endogenous metabolites such as 7 $\beta$ -OH-DHEA/DHEA and 16 $\alpha$ -DHEA/DHEA were not significantly different after administration of different inhibitor and inducer. In conclusion, endogenous metabolites such as 6 $\beta$ -OH-cortisol/cortisol and 6 $\beta$ -OH-cortisone/cortisone seem to be more sensitive in phenotyping CYP3A4 activity reflecting the extents of inhibition and induction than midazolam clearance.

**Poster #: 437**

**Abstract #: 2522**

**Abstract Title:** LC-MS QTOF lipidomics approach of faecal samples in IBD patients

**Authors:** Antonio Murgia, Sonia Liggi, Maria Santoru, Cristina Piras, Tonina Lai, Paolo Usai, Francesca Boi, Aldo Manzin, Luigi Atzori, Pierluigi Caboni,

**Presenting Author Affiliation:** University of Cagliari

**Abstract Submission:**

Although in the recent past the incidence of Inflammatory Bowel Diseases (IBD) has noticeably increased, its etiology is still unclear. No specific pathogen has been defined as a causative agent. Serological biomarkers were been recently proposed for diagnosis, but they remain unused in clinical applications. Moreover, current diagnostic and monitoring practices for IBD are very invasive. Therefore, accurate tools for early diagnosis and in particular non-invasive strategies are needed. In this context, metabolomics could represent a useful approach to understand possible pathological mechanisms of action or metabolites modification in different pathways. In this work, the lipid metabolite pool of fecal samples of 132 patients affected by IBD and 51 of healthy patients has been studied by high resolution liquid chromatography coupled to a time of fly mass spectrometry (LC-MS Q-TOF) and multivariate statistical data analysis. By these means, differences between Crohn, ulcerative colitis and control samples were investigated. Results of discriminant analysis were considered with the aim of finding the relevant metabolites unique for each class. The results highlight differences in the lipid metabolite profile between the pathological and the control samples. In particular, the most discriminant metabolites for both IBD classes were a member of the family of acyl trehalose and a compound belonging to the class of cholecalciferols. The first one is a saccharolipid precursor of the acetylated glucosamine, a lipid A constituent of GRAM – such as *Escherichia coli*, while the second one is a precursor of vitamin D3. However, no differences were found between the two pathological classes. These results highlight similarity in the metabolic alteration occurring in both ulcerative colitis and Chron disease. In conclusion, the application of metabolomics to fecal samples of IBD allows identifying possible metabolites that can be used as a indicators of metabolic pathways implicated on the onset of these pathologies.

**Poster #: 438**

**Abstract #: 2607**

**Abstract Title:** Metabolome analysis of perioperative serum and urine in hepatectomy patients with hepatocellular carcinoma

**Authors:** Hisami Yamanaka-Okumura, Akiyoshi Hirayama, Daisuke Kajiura, Hiroshi Tatano, Mitsuo Shimada, Masaru Tomita, Tomoyoshi Soga,

**Presenting Author Affiliation:**

**Abstract Submission:**

Perioperative nutritional management is an essential factor for early recovery after surgery. We aimed to examine the specific changes of serum and urine metabolites before and after hepatectomy.

Patients with hepatocellular carcinoma (n = 16, Age  $67 \pm 2$  y, M13 / F3, BMI  $22.2 \pm 0.6$  kg/m<sup>2</sup>) were recruited in this study. Etiologies of the disease varied as followed: 6HBV, 4HCV, 2alcoholic, 1HCV+alcoholic, and 3others. Serum (S) and spot urine (U) were collected before (S0, U0) and after 1 (S1), 3 (S3, U3), 14 (S14) days of hepatectomy at fasting state, respectively. Blood samples were used to measure the levels of WBC, CRP, PLT, AST, ALT, T-Bil and Alb. Metabolites in serum and urine were analyzed by CE-TOFMS. Insulin levels in S1 and S3 were significantly higher than that in S0. Postoperative serum (S1, S3, S14) and urine (U3) metabolites were compared with baseline levels (S0 or U0).

In S1, BCAA (Val, Leu, Ile) levels tended to decrease and AAA (Tyr, Phe) levels significantly increased, then Fischer ratio significantly decreased after liver resection. Increment of serum taurine levels, which major component of skeletal muscle, was correlated with WBC in S1 and S3, and ALT in S14 by multivariate regression analysis. Inflammation after liver resection showed to accelerate muscle breakdown. In this study, dynamic changes of metabolites at day 1 and day 3 were shown and catabolism of skeletal muscle was recovered at day 14. These data suggested that intensive nutritional care was required at preoperative and early postoperative periods.

Metabolome analysis by CE-TOFMS was useful tool for perioperative assessment.

**Poster #: 439**

**Abstract #: 2498**

**Abstract Title:** Spatial gut differences and cross-species comparison of gut metabolite profiles via metabolite profiling analysis

**Authors:** Silke Heinzmann, Philippe Schmitt-Kopplin,

**Presenting Author Affiliation:** Helmholtz Zentrum München

**Abstract Submission:**

Knowledge of presence of biologically active compounds in tissues and biofluids is of crucial importance to draw conclusions on potential ways to influence metabolism in health and disease. While microbiome research has done great progress in locating specific bacteria in distinct gut segments, knowledge on the metabolome is sparse. To date, most studies have focused on the analysis of feces as the gut's end product, omitting the specialized metabolic and physiological functions of different sections of the gut. NMR spectroscopy profits from lack of inter-metabolite suppression effects, which enables a parallel investigation of different sample matrices and the quantitative nature of analysis. We report spatial variation of gut luminal metabolites in mice that directly link to microbial breakdown of carbohydrates and proteins in the cecum, re-absorption processes in the colon and show the de-hydroxylation and deconjugation pattern of bile acids along the gut. Since the translatability of rodent models to human health and disease is limited, we extend our investigation to other animal models. Spatial and cross-species metabolic variation is affected by the microbiota but might also directly promote the feedback for growth of selected bacteria along the gut and trigger signaling pathways for the microbiome and the host.

**Poster #: 440**

**Abstract #: 2541**

**Abstract Title:** Metabolomic changes during inhibition of histone demethylase in murine embryonic stem cells

**Authors:** Sven Zukunft, Timo Frömel, Ingrid Fleming,

**Presenting Author Affiliation:** Goethe University Frankfurt

**Abstract Submission:**

Recent studies showed a connection between metabolism and histone methylation dynamics. Several components of epigenetic machinery require intermediates of the cellular metabolism as co-factors. Furthermore, changes in cellular metabolic homeostasis can alter expression and enzymatic function of histone methyltransferases.

In this study we investigate the influence of the lysine-specific histone demethylase 1 (LSD1) on cellular metabolic homeostasis. LSD1 belong to a family of FAD dependent histone lysine demethylases (KDMs), acting as transcriptional coactivator or corepressor by demethylation of histone H3. Therefore, LSD1 has been implicated as a potential therapeutic target in cancer and other disease.

To investigate which biochemical pathways are affected by LSD1, murine embryonic stem cells were treated 4 days with the LSD1 inhibitor GSK-LSD1. Metabolites were extracted from the cells in two steps, allowing the simultaneously detection of hydrophilic and lipophilic compounds. Therefore, metabolites were extracted from the cells with 80% methanol and the remaining pellet was extracted with ethyl acetate. Both extracts were evaporated, re-dissolved in 20% methanol and combined. These samples were analyzed by LC-MS. Chromatographic separation was performed on a HSS T3 C18 column. Mass spectrometry was done using a high resolution accurate mass (Orbitrap) mass spectrometer. Data were analyzed with XCMS to determine differently regulated metabolites.

Principal component analysis on all detected features clearly separate the LSD inhibitor treated from the untreated group. Interestingly, some of the prominent changes occurred for metabolites which are related to epigenetic modifications. The polyamines spermine and spermidine, both themselves directly influencing chromatin structure were found to be decreased. Additionally,  $\alpha$ -ketoglutarate which is an essential cofactor for several enzymes including other KDMs, were found to be decreased during LSD1 inhibitor treatment. Our results indicated a feedback loop between epigenetic regulation and homeostasis of metabolites acting as cofactors in the epigenetic regulation.



**Poster #: 441**

**Abstract #: 2212**

**Abstract Title:** Valid assessment of sample quality, the crucial factor for reliable outcomes in clinical metabolomics studies

**Authors:** Rainer Lehmann, Xinyu Liu, Miriam Hoene, Peiyuan Yin, Guowang Xu,

**Presenting Author Affiliation:** Division of Clinical Chemistry and Pathobiochemist

**Abstract Submission:**

High resolution mass spectrometry approaches are one mainstay of clinical metabolomics. Another even more important, but less considered key success factor is the quality of blood samples used, since conclusions drawn from clinical metabolomics studies can heavily be biased by preanalytical variabilities and cannot be overcome by high resolution mass spectrometry. Up to the present there are no general accepted valid tools to directly assess the quality of serum or plasma samples. The aim of this study was to identify and rigorously validate a quality marker applicable for high throughput assessment of clinical and biobank samples.

Applying non-targeted metabolomics we detected a biomarker suitable for the assessment of serum and plasma quality without having knowledge of the preceding processing of the blood. This biomarker was rigorously validated by targeted UPLC-MS analyses including evaluation of the robustness using 650 samples and various conditions. The definition of confidence intervals enables us to classify blood samples into excellent, good (4 h exposure to RT). Finally, the quality of 500 spot samples from different international biobanks were checked thereby uncovering disillusioning discrepancies between prescribed operating procedures and the related sample quality in a considerable number of cases.

To conclude, the quantification of this novel quality control metabolite biomarker offers a unique possibility for a valid assessment of the quality of serum and plasma samples pre- and post-storage thereby allowing the selection of solely excellent samples for clinical metabolomics. This strategy can significantly improve the outcome of clinical metabolomics and beyond.

**Poster #: 442**

**Abstract #: 2234**

**Abstract Title:** Nuclear magnetic resonance metabolomic profiling of mouse kidney, urine and serum following renal ischemia/reperfusion injury

**Authors:** Justine Leenders, François Jouret, Laurence Poma, Jean-Olivier Defraigne, Jean-Marie Krzesinski, Pascal de Tullio,

**Presenting Author Affiliation:** Ulg (CIRM)

**Abstract Submission:**

Ischemia/reperfusion (I/R) is the most common cause of acute kidney injury(AKI). Its pathophysiology remains unclear. Metabolomics is dedicated to identify metabolites involved in (patho)physiological changes of integrated living systems. Here, we performed <sup>1</sup>H-Nuclear Magnetic Resonance metabolomics using urine, serum and kidney samples from a mouse model of renal I/R.

Renal 30-min ischemia was induced in 12-week-old C57BL/6J male mice by bilaterally clamping vascular pedicles, and was followed by 6, 24 or 48-hour reperfusion. Sham-operated mice were used as controls. Statistical discriminant analyses (DA), i.e. principal component analysis and orthogonal projections to latent structures, were performed on urine, serum and kidney lysates at each time-point. Multivariate receiver operating characteristic (ROC) curves were drawn, and sensitivity and specificity were calculated from ROC confusion matrix.

Urine DA showed a net separation between I/R and sham groups, with significant variations in levels of taurine, di- and tri-methylamine, creatine and lactate. Such changes were observed as early as 6 hours post reperfusion. Major metabolome modifications occurred at 24h post reperfusion. At this time-point, correlation coefficients between urine spectra and conventional AKI biomarkers, i.e. serum creatinine and urea levels, reached 0.94 and 0.95, respectively. The area under ROC curve at 6h, 24h and 48h post-surgery were 0.73, 0.98 and 0.97, respectively. Similar discriminations were found in kidney samples, with changes in levels of lactate, fatty acids, choline and taurine. By contrast, serum DA could not discriminate sham-operated from I/R-exposed animals.

Our study demonstrates that renal I/R causes early and sustained metabolic changes in urine and kidney composition. The most implicated pathways at 6h and 24h post reperfusion include gluconeogenesis, taurine and hypotaurine metabolism, whereas protein biosynthesis, glycolysis, and galactose and arginine metabolism are key at 48h post reperfusion. These data open new research avenues to better understand, diagnose and prevent renal I/R.

**Poster #: 443**

**Abstract #: 2283**

**Abstract Title:** 5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR): A concurrently causative endogenous metabolite of Lesch-Nyhan disease?

**Authors:** Sarah Tschirner, Heike Bähre, Alexander Kaever, Erich Schneider, Roland Seifert, Volkhard Kaever,

**Presenting Author Affiliation:** Institute of Pharmacology, Hannover Medical School

**Abstract Submission:**

5-Aminoimidazole-4-carboxamide ribonucleotide (AICAR): A concurrently causative endogenous metabolite of Lesch-Nyhan disease?

Tschirner SK(1), Bähre H(1,2), Kaever A(3), Schneider EH(1), Seifert R(1), Kaever V(1,2)

(1)Institute of Pharmacology, Hannover Medical School, Hannover, Germany

(2)Research Core Unit Metabolomics, Hannover Medical School, Hannover, Germany

(3)Department of Bioinformatics, Institute of Microbiology and Genetics, Georg-August-University, Göttingen, Germany

The rare disorder Lesch-Nyhan disease (LND) is characterized by hyperuricemia, neurological symptoms, and behavioral abnormalities such as repetitive self-mutilation. LND is caused by congenital deficiency of the purine-salvage enzyme hypoxanthine-guanine phosphoribosyltransferase (HPRT) due to various mutations of the X-chromosomal HPRT1 gene. So far, no causal therapy is established for attenuation of the neurological and behavioral manifestations.

Applying a non-targeted metabolomics approach via HPLC-coupled quadrupole time-of-flight mass spectrometry, we performed a hypothesis-free metabolite screening of brain extracts from HPRT knockout (HPRT KO) mice as established animal model for LND. Peak finding and alignment as well as principal component analysis were performed by MarkerView™ Software (Sciex). For subsequent biomarker evaluation the software MarVis-Suite 2.0 (<http://marvis.gobics.de>) was used.

Nearly 20,000 peaks were detected by our method for brain extracts. About 10% of these features passed quality control criteria. Nine monoisotopic features were found in significantly different abundancies for HPRT KO compared to wild type mice. After mapping to compound databases and confirmation by the authentic standard substance, 5-aminoimidazole-4-carboxamide ribonucleotide (AICAR) was significantly increased in the brains of HPRT KO mice.

This result fits well with recently published data showing AICAR accumulation in erythrocytes of LND patients [Ceballos-Picot I et al., Orphanet J Rare Dis (2015) 10:7] implying a causative relationship between elevated AICAR concentrations and neurological defects. As AICAR is known as activator of AMP-activated kinase (AMPK), it should be contemplated to initiate clinical trials with AMPK inhibitors such as dorsomorphin, ideally starting before the onset of severe clinical symptoms.

**Poster #: 444**

**Abstract #: 2393**

**Abstract Title:** Liquid Chromatography-Mass Spectrometry and Chemometric analysis of *Ricinus communis* extracts for cultivar identification

**Authors:** Simon Ovenden, Eloise Pigott, Simone Rochfort, David Bourne,

**Presenting Author Affiliation:** DST Group

**Abstract Submission:**

Seeds of *Ricinus communis* contain the toxic protein ricin, a 64kD heterodimeric type II ribosome-inactivating protein which has been used in several high profile incidents. The ability to determine what cultivar the toxin was isolated from via an LCMS method would be of significant use to law enforcement and forensic agencies.

To this end, seeds from eight specimens of six cultivars of *Ricinus communis* ("carmencita", "dehradun", "gibsonii", "impala", "sanguineus" and "zanzibariensis") were extracted using a standard methodology, analysed by LC-MS and subjected to Chemometric analysis (PCA and OPLS-DA). Identified compounds of importance were subjected to HRFTMS and MS/MS to elucidate their structures. This analysis identified 17 ions as potential cultivar determinators. Through accurate mass measurement and MS/MS, molecular formulas for 13 ions were determined, including two known and 11 new peptides.

From this analysis, unique ions from extracts of "carmencita", "dehradun", "gibsonii", "impala", and "zanzibariensis" were identified that would allow an individual cultivar to be distinguished from other cultivars in this study. While "sanguineus" extracts contained no unique compounds, a unique LC-MS profile would allow for cultivar assignment.

**Poster #: 445**

**Abstract #: 2596**

**Abstract Title:** Whole Transcriptome Integration with Metabolome Identifies Unique Metabolic Strategies in *Pseudomonas aeruginosa* Biofilms

**Authors:** Valerie Copie,

**Presenting Author Affiliation:** Montana State University

**Abstract Submission:**

Considered independent, free-living single cell organisms, most bacteria in natural environments exist as organized communities of cells called biofilms. Despite many years of research, there remains much unknown about the metabolic phenotype of the biofilm mode of growth. Defined as structured communities of cells adhered to a surface, biofilms exhibit significant phenotypic heterogeneity and bacterial cells must not only contend with limited nutrient and oxygen availability, but must also employ strategies to cope with high levels of waste products, secondary metabolites, and secreted factors. *Pseudomonas aeruginosa* is a Gram-negative, aerobic bacterium able to survive in diverse biotic and abiotic environments. In the clinic, *P. aeruginosa* notoriously causes serious infections in cystic fibrosis lungs and chronic wounds. Success of *P. aeruginosa* in such diverse environments is due in part to a large and complex genome, as well as an adaptive ability to switch between the biofilm and planktonic mode of growth. Much remains to be understood about the molecular basis of planktonic and biofilm metabolism in *P. aeruginosa*. Here we utilize a biofilm model where biofilms are grown at a liquid – surface – air interface mimicking the complex nutrient environment of a seeping wound. Because biofilm development is dependent on availability of organic nutrients, iron, and oxygen and resistance of biofilms to antibiotics is associated with oxygen limitation, it is important to assess metabolic phenotype in a model reflective of *P. aeruginosa* growth in the clinical setting. We demonstrate the metabolite profiles that distinguish the biofilm and planktonic phenotypes in *P. aeruginosa* and integrate transcriptomic profiles into the metabolomic profiles to gain a more comprehensive understanding of the fundamental molecular mechanisms that contribute to the metabolic versatility of this opportunistic pathogen with the long term goal of identifying metabolic strategies that might be targeted for therapeutic intervention.

**Poster #: 446**

**Abstract #: 2018**

**Abstract Title:** The muscle metabolome differs between healthy and frail older adults

**Authors:** parastoo Fazelzadeh,

**Presenting Author Affiliation:**

**Abstract Submission:**

A major contributor to the frailty syndrome of ageing is loss of skeletal muscle, which negatively affects quality of life in the elderly. As a crucial metabolic organ in the body, skeletal muscle distinguishes itself by significant phenotypical plasticity and the ability to influence important metabolic events elsewhere in the body. Clear insight in how phenotypical differences and plasticity of healthy muscle are reflected in the muscle metabolome is currently lacking, mainly due to lack of metabolic profiling capability.

Hence we developed a comprehensive extraction procedure for muscle biopsies that in combination with suite of targeted MS-based profiling platforms, provides broad coverage of organic acids, amines, nucleotides, acylcarnitines and oxylipins. We assessed the muscle biopsy metabolome of young (n=30), healthy older (n=65) and frail older (n=44) subjects to determine the effect of ageing and frailty on the metabolic signature of muscle tissue. Moreover, effects of whole-body resistance-type exercise training on the muscle biopsy metabolome of older subjects were examined. Young subjects were included as a reference for expected shifts of the older towards younger metabolic phenotype. The major differences in muscle metabolome of healthy older and young subjects relate to mitochondrial function, fiber-type composition, and tissue turnover. Similar differences were observed comparing frail with healthy older subjects. Prolonged resistance-type exercise training showed a correlative adaptive response of amino acids and genes responsible for tissue remodeling. The effect of exercise on amino acid derived acylcarnitines in older subjects points towards decreased branched chain amino acids catabolism likely due to attenuated activation of the flux-determining mitochondrial branched chain  $\alpha$ -keto acid hydrogenase complex in older subjects. Only modest correlations between muscle metabolite and plasma levels were found, which prohibits the use of the latter as read-outs of muscle metabolism.

**Poster #: 448**

**Abstract #: 2102**

**Abstract Title:** Metabolic risk markers for the development of Celiac Disease: Results of the PreventCD study

**Authors:** Franca Kirchberg, Katharina Werkstetter, Olaf Uhl, Renata Auricchio, Gemma Castillejo, Ilma Korponay-Szabo, Isabel Polanco, Carmen Ribes Koninckx, Sabine Vriezinga, Berthold Koletzko, Maria Luisa Mearin, Christian Hellmuth,

**Presenting Author Affiliation:** LMU Munich

**Abstract Submission:**

**Objectives and study:** The mechanisms involved in the development of celiac disease (CD) are still not completely understood. Previous studies found that the serum and urine metabolic profiles differed between healthy individuals and CD patients. We test the hypothesis of a specific predictive molecular profile in 4 month old infants with future CD diagnosis.

**Methods:** Serum samples were available for 230 children of the PreventCD study, a multicenter, randomized, double-blind, dietary intervention study. All children were positive for HLA-DQ2 and/or HLA-DQ8 and had at least one first-degree relative diagnosed with CD. Twenty-two amino acids were quantified after derivatization with liquid chromatography – tandem mass spectrometry (MS/MS), and 145 phospholipids as well as 16 acylcarnitine concentrations were determined with direct infusion MS/MS. We investigated the association of the metabolic profile in the 4-months old infants with the development of CD until school age (yes/no). All analyses were repeated in a subsample of 174 exclusively breastfed infants.

**Results:** By the end of 2014, twenty-three (subsample: 22) out of the 230 (subsample: 174) children were diagnosed with CD according to the ESPGHAN criteria. Testing each metabolite for a different concentration between healthy and CD children we found that the phosphatidylcholines were the most striking species. This result was strongest in the exclusively breastfed infants. However, neither a single phosphatidylcholine nor their sum reached statistical significance.

**Conclusion:** Our findings do not support a relation of the metabolic profile at age of 4 months prior to gluten-consumption with respect to a later CD development. To understand and prevent the development of CD, we suggest to analyze the metabolic profile at a later time point after the introduction of gluten.

**Poster #: 449**

**Abstract #: 2115**

**Abstract Title:** Molecular Abnormalities in Schizophrenia: Neurotransmission, Oxidative Stress, and Apoptosis

**Authors:** Elizabeth Want, Hendrik Wesseling, Elaine Holmes, Sabine Bahn,

**Presenting Author Affiliation:** Imperial College London

**Abstract Submission:**

The neuropsychiatric disorder schizophrenia affects 1% of the world's population. It manifests with a broad, heterogeneous range of symptoms, hindering the effectiveness of current therapies. New models capable of reproducing core pathological features of schizophrenia are needed to elucidate pathological disease mechanisms, identify biomarkers for improved diagnosis and discover potential novel drug targets.

Metabolic profiling using ultra performance liquid chromatography-mass spectrometry (UPLC-MS) was combined with comprehensive global label-free and targeted proteomics (selected reaction monitoring and multiplex-immunoassay) on serum and brain from a chronic phencyclidine (PCP) rat model. Here, NMDA-receptor hypofunction is induced through non-competitive NMDAR-receptor antagonism. Molecular profiling of serum was performed to improve understanding of associated systemic effects, as PCP modulates immunoregulatory function. A trend toward an anti-inflammatory state was observed, with alterations in cytokine levels (IL-5, IL-2, IL-1 $\beta$ ) and fibroblast growth factor-2.

Chronic PCP treatment had a larger effect on the hippocampal proteome and metabolome compared to the frontal cortex. Metabolic profiling of frontal cortex and hippocampal samples revealed changes in lipid metabolism, particularly glycerophospholipids. This was further supported by findings of oxidative stress through altered superoxide dismutase levels, indicative of apoptotic pathway alterations. Bioinformatic pathway analysis confirmed abnormalities in NMDA-receptor associated pathways in both brain regions, as well as alterations in Kainate, AMPA and GABAergic signalling in the hippocampus. These findings were also correlated with hippocampal behavioural functions.

These molecular changes parallel findings observed in human schizophrenia, where changes in hippocampal function have been linked to schizophrenia, opening up new avenues of research, as previous studies focused on potential frontal cortex abnormalities. This study could lead to increased understanding of how perturbed glutamate receptor signalling affects other relevant biological pathways in schizophrenia. This may lead to the discovery of potential novel drug targets for improved treatment, while surrogate markers in blood can be translated to the clinic.



**Poster #: 450**

**Abstract #: 2308**

**Abstract Title:** MALDI imaging in kidney for lipidomics studies of cisplatin-induced renal damage and cilastatin nephroprotection assessment

**Authors:** Estefanía Moreno-Gordaliza, Diego Esteban-Fernández, Alberto Lázaro, Blanca Humanes, Sarah Aboulmagd, Alberto Tejedor, Michael Linscheid, Milagros Gómez-Gómez,

**Presenting Author Affiliation:** Universidad Complutense de Madrid

**Abstract Submission:**

The most limiting side effect of cisplatin-based antitumor therapies is nephrotoxicity. The drug has been reported to accumulate in the kidney cortex and corticomedullary junction [1], inducing proximal tubule cells damage. Cilastatin is able to significantly reduce this accumulation and has been demonstrated to act as a nephroprotector for these therapies [2]. However, the molecular mechanisms for cisplatin renal damage and cilastatin renoprotection are still not completely understood.

A method for sensitive and reproducible lipid imaging by MALDI-Orbitrap MSI has been successfully developed and applied to kidney sections from rats under cisplatin treatment. Matrix deposition for positive and negative lipid ions imaging using 9-aminoacridyne, 2,5-dihydroxibenzoic acid or alpha-cyano-4-hydroxycinnamic acid has been investigated for best results. Reproducibility of normalized images from parallel tissue sections prepared and analyzed over time was demonstrated. The effect of the drug on the renal rat lipidome was studied, allowing detection of alterations in lipids distribution within renal substructures during induced nephrotoxicity, in comparison with control healthy animals. The combination of positive and negative imaging modes showed 71 different lipids (mostly phospholipid species) to be altered by cisplatin. On the other hand, co-administration of the drug with cilastatin resulted in renoprotection and significant preservation of the control healthy lipid species distribution. Cisplatin-altered lipid species patterns reflect either topographic and structural changes or signaling processes taking place in the damaged kidney.

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**Poster #: 451**

**Abstract #: 2431**

**Abstract Title:** A METABOLITE PROFILING APPROACH TO DISCRIMINATE AND CLASSIFY SWEET CHERRIES ACCORDING TO THEIR GEOGRAPHICAL ORIGIN BY HPLC-ESI-QTOF-MS AND MULTIVARIATE DATA ANALYSIS

**Authors:** Rosa Quirantes-Piné, Celia Rodríguez-Pérez, Antonio Segura-Carretero, Alberto Fernández-Gutiérrez,

**Presenting Author Affiliation:** University of Granada

**Abstract Submission:**

Metabolomic approaches have proved to be valuable in a wide range of areas of knowledge, including diseases diagnostics, drug discovery, nutrition and food science, where it has been used for discriminative and predictive purposes associated with food quality and safety.

In the present study, a metabolite profiling approach was applied for the discrimination and classification of sweet cherries from different geographical origins using liquid chromatography quadrupole-time of flight mass spectrometry (HPLC-ESI-QTOF-MS) and multivariate analysis. Fifty nine sweet cherry samples from two regions, Valle del Jerte and Güejar Sierra, were selected to assess the developed procedure consisting of untargeted metabolite profiling by HPLC-ESI-QTOF-MS, application of an automated molecular features extraction algorithm followed by multivariate statistical analysis for the detection of discriminative metabolites and finally, biomarkers identification. A commercial software package was used for data mining including extraction of input variables, retention times alignment, normalization, scaling and multivariate statistical analysis to obtain a predictive model for discrimination of sweet cherry samples according to their geographical origin. Furthermore, the potential of QTOF-MS for the putative identification of biomarkers characteristic of each origin has been proved combining high sensitivity and mass accuracy for both precursor and product ions, providing the elemental composition of the parent and fragment ions.

This study brings to light the potential of MS-based metabolite profiling in combination with chemometric tools for discriminating and classifying food products.

**Poster #: 452**

**Abstract #: 2465**

**Abstract Title:** Characterising a core metabolic enzyme responsible for phosphine resistance and fundamental metabolic regulation.

**Authors:** Horst Joachim Schirra, Paul Ebert,

**Presenting Author Affiliation:** The University of Queensland, Centre for Advanced

**Abstract Submission:**

Phosphine is a small redox-active gas that is used to protect global grain reserves from pest insects, but these insects are increasingly resistant to phosphine fumigation. Recently, 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 (Schlipalius et al., Science, 2012, 338:807 – 810). DLDH is a core metabolic enzyme, central to metabolic regulation, and a new class of resistance factor. Polymorphisms responsible for genetic resistance cluster around the redox-active catalytic disulfide or the dimerisation interface of DLDH in insects and nematodes. DLDH participates in four key steps of core metabolism, which are affected differently by phosphine exposure 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, aerobic and anaerobic respiration. Metabolomic analysis indicates a role of DLDH in the cross-talk between branched-chain amino acid and lipid metabolism. A genome-scale metabolic model of *C. elegans* metabolism is being developed that will enable further characterization of this metabolic link. DLDH is an exceptional case in which a combination of systems biology methods has identified a single genetic cause of phenotypic change that is responsible for metabolic adaptation in health and disease and that can subsequently be studied with a wide range of methods ranging from structural biology, genetics, and classical biochemistry to systems biology and metabolic modelling.

**Poster #: 453**

**Abstract #: 2574**

**Abstract Title:** Brain metabolome study during neurodevelopment in a rat model of autism

**Authors:** Binta Dieme, Lefevre Antoine, Sylvie Chalon, Laurent Galineau, Helene Blasco, Denis Guilloteau, Christian Andres, Lydie Nadal-Desbarats, Patrick Emond,

**Presenting Author Affiliation:** INSERM

**Abstract Submission:**

Autism (ASD) belongs to neurodevelopmental disorders that persist throughout life and includes disorders of social interactions, communication deficits and restricted and stereotyped behaviors. The pathophysiology of autism remains unknown, and to date, there are no biomarkers available for diagnosis or therapeutic monitoring.

The proposed study aims to explore the metabolome of different brain regions during development in a rat model of ASD. This model was obtained by exposing pregnant rats to valproic acid (VPA) on the twelfth day of gestation. Brain metabolome (cerebellum, frontal and parietal cortex) was explored by LC-HRMS at two stages of development (adolescent P21, n=19 and adult P35, n=16).

For each brain regions, about one hundred and thirty metabolites were identified through an in house database obtained from standards injected using identical analytical methods.

First, our results showed an effect of brain development on the metabolome in each region of interest defining a metabolic trajectory (Cerebellum  $R^2X[1]=0.27$ ,  $R^2X[2]=0.17$ , parietal cortex  $R^2X[1]=0.31$ ,  $R^2X[2]=0.17$ , frontal cortex  $R^2X[1]=0.26$ ,  $R^2X[2]=0.13$ . In fact, VPA rats display specific abnormalities related to metabolic pathways involving phenylalanine, tryptophan, tyrosine, purines and pyrimidines, cysteine, methionine and fatty acids.

Moreover, a specific effect due to the prenatal VPA treatment was also observed when we looked for the metabolic differences at each developmental stage. We found a decrease in GABA in the cerebellum and frontal cortex, which supports the hypothesis of an imbalance excitation / inhibition in ASD. In the three regions of the brain that we have explored, a potential disruption of neurotransmission, oxidative stress, and metabolism of amino acids, purines and pyrimidines has also been found.

**Poster #: 454**

**Abstract #: 2000**

**Abstract Title:** Metabolomics and Lipidomics Reveal a Pro-inflammatory Response to Ionizing Radiation

**Authors:** Evagelia Laiakis, Albert Fornace Jr, Giuseppe Astarita,

**Presenting Author Affiliation:**

**Abstract Submission:**

The identification of cancer patients who might develop severe adverse reactions in response to radiotherapy has been hindered by the complexity of individual variation in sensitivity to radiation. The molecular response to ionizing radiation, however, is still not completely understood. Here we screened mouse serum for metabolic alterations following an acute exposure to gamma radiation using a multi-platform, mass-spectrometry-based strategy. A global, metabolomics and lipidomics profiling allowed to screen and identify alterations in selected metabolites. A targeted approach allowed to expand the initial observation monitoring the effects of radiation exposure on key biochemical pathways. Exposure to gamma radiation induced a significant increase in the serum levels of ether phosphatidylcholines (PCs) while decreasing the levels of diacyl PCs carrying PUFAs. In exposed mice, levels of pro-inflammatory, oxygenated metabolites of arachidonic acid increased, whereas levels of anti-inflammatory metabolites of omega-3 PUFAs decreased. The obtained molecular biosignature might be used as an indicator of radiation exposure and, potentially, as a predictor of radiosensitivity. Verification studies are currently undergoing in human samples. If validated, baseline levels of eicosanoids (e.g., omega-6/omega-3 ratio) might serve as a companion diagnostic tool for radiation therapy, to help differentiate cancer patients who would respond best to radiotherapy treatment from radiosensitive patients, who may be unable to tolerate the additional inflammatory response induced by radiotherapy. Most importantly, the ability to control eicosanoids pathways with pharmacological or dietary interventions (i.e., omega-3 supplementation) might alleviate and eventually offset many of the side effects linked to radiation therapy.

**Poster #: 455**

**Abstract #: 2127**

**Abstract Title:** Ageing muscle metabolism: The effect of exercise on muscle during the adult lifecourse

**Authors:** Warwick Dunn, Daniel Wilkinson, Bethan Phillips, Giovanni Rodriguez-Blanco, Andrew Chetwynd, Kenneth Smith, Philip Atherton,

**Presenting Author Affiliation:** School of Biosciences and Phenome Centre Birmingham

**Abstract Submission:**

Introduction: Poor musculoskeletal health in adult life has a significant impact upon quality of life, work productivity and health costs around 10 million working days were lost in the UK through musculoskeletal conditions in 2006/7 with an annual cost to the NHS related to musculoskeletal decline at £5.7 billion. The degenerative loss of skeletal muscle mass, quality and strength (sarcopenia) is often associated with old age and frailty though starts to occur from age 30 in most adults. Poor musculoskeletal health has an impact on the older population where retirement ages and lifespan are increasing there is a requirement for healthspan to closely match lifespan to ensure that years of low mobility or disability are not observed in later life. The two greatest factors in the development of musculoskeletal pathology are age and obesity.

Objectives and Methods: In this study we have dissected the influence of age and exercise status on the metabolic composition of muscle. Muscle from three age groups (18-30y N=11, 45-55y N=20 and 65-75y N=20) was sampled at (1) basal, and (2) acutely post fed and exercise both prior to and following 20 weeks of supervised whole body resistance exercise training. Following Folch extraction the lipophilic and hydrophilic extracts were separately analysed applying HILIC UHPLC-MS and C18 reversed phase UHPLC-MS non-targeted metabolomics with the data analysed applying univariate and multivariate analysis methods. Associations between metabolite concentrations and clinical endpoints (e.g. HOMA-IR and hand grip) were also investigated.

Results and conclusions: Exercise and fed/fasted state contributed to changes in the muscle metabolome with class-specific changes observed in hydrophilic and lipophilic metabolite classes. The results and their impact on musculoskeletal health will be discussed.

**Poster #: 456**

**Abstract #: 2479**

**Abstract Title:** Metabolomics Approaches on Canadine Cytochrome P450 Reaction Phenotyping

**Authors:** Xuejun Peng, Ulrike Schweiger Hufnagel,, Aiko Barsch,

**Presenting Author Affiliation:** Metabolomics Society

**Abstract Submission:**

Cytochrome P450 (CYP) exhibits polymorphic expression in human populations and contributes to variable exposure levels for drug metabolism. Reaction phenotyping as the process to identify specific pathway of in vitro drug-metabolizing enzymes involved in drug clearance has been an initial strategy in drug discovery or early drug development stage to provide early insight on potential drug-drug interactions or possible affinities to functionally polymorphic enzymes which can cause inter-subject variability. Common experimental approach for CYP reaction phenotyping includes incubations with cDNA expressed CYP followed by the relative quantitation of substrate depletion, an imperfect approach due to limited availability of radiolabeled drug or synthetic metabolite standards. In this application, a novel LC-QTOF metabolomics approach to systemically study the reaction phenotyping of a protoberberine alkaloid Canadine was performed by simultaneously detecting the drug depletion and all metabolites formation. cDNA expressed human supersomes isoforms CYP1A2, 2A6, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4 and controls were spiked into a pre-incubated NADPH regenerating reaction system incubated at 37 °C for 60 minutes. The reactions were stopped by cold acetonitrile followed by centrifugation. Six replicates of supernatant were injected in an UPLC interfaced with a Bruker QTOF for separation and detection. Data were processed by DataAnalysis, ProfileAnalysis and Metaboscape software for automatic mass calibration, statistical analysis, metabolites profiling and identification. Major Canadine metabolites by O-demethylation, hydroxylation or N-oxidation, methylenedioxy bridge opening, the combination of O-demethylation and hydroxylation were investigated based on PCA and structural elucidation. CYP2C9, 2C19 and 2D6 were identified for all metabolites, O-demethylation and methylenedioxy bridge opening metabolites were produced by all tested CYPs. The relative quantitation of Canadine depletion and metabolites formation related to each of the CYP isoforms were calculated and outlined. The reaction phenotyping for Canadine by metabolomics approach was proposed.

**Poster #: 457**

**Abstract #: 2136**

**Abstract Title:** Induced sputum lipidomics in the study of lung diseases

**Authors:** Ruben t'Kindt, Eef Telenga, Roland Hoffman, Antoon van Oosterhout, Nick ten Hacken, Pat Sandra, Koen Sandra,

**Presenting Author Affiliation:** RIC

**Abstract Submission:**

The present contribution reports on the application of a lipidomics platform in the study of lung diseases. Lipids extracted from induced sputum samples of smokers with and without chronic obstructive pulmonary disease (COPD) were subjected onto a lipidomics method combining high resolution reversed-phase liquid chromatography (RP-LC) with high resolution quadrupole time-of-flight mass spectrometry (Q-TOF). Differential and non-differential lipids were identified based on accurate mass, retention time and MS/MS and were compiled in an accurate mass and retention time library (AMRT). Using the lipidomics method, over 1,500 lipids including isomers, originating from 6 different lipid classes (fatty acids, phospholipids, sphingolipids, prenol lipids, sterol lipids, glycerolipids), have been accurately identified in 120  $\mu$ L induced sputum. Furthermore, lipid screening was performed on 39 lung sputum samples in order to study the effect of COPD on the lipidome. The study shows that lipid expression in induced sputum significantly differs between smokers with (n=19) and without (n=20) COPD. Major changes in the sphingolipid pathway are observed with a large fraction (168 species) of ceramides, sphingomyelins and more complex glycosphingolipids being significantly upregulated in the COPD sample set. Of interest, ceramides, dihydroceramides and phytoceramides with saturated fatty acid side chains showed an increase in COPD that was dependent on fatty acid chain length. As such, lipidomics allows to investigate both the behavior of lipid classes and individual lipid species. All findings were validated in an independent sample set which also investigated the effect of 2 month smoking cessation. For future purposes, recognizing and understanding the altered (sputum) lipidome might prove beneficial for more accurate diagnostics and possibly better treatment.



**Poster #: 458**

**Abstract #: 2140**

**Abstract Title:** Differential Complex Lipid Plasma Profiles in Women with Preeclampsia

**Authors:** Baljit Ubhi, Susan Fisher, Katherine Williams,

**Presenting Author Affiliation:** SCIEX

**Abstract Submission:**

Preeclampsia, the onset of hypertension and proteinuria at  $\geq 20$  weeks of gestation, is a leading cause of maternal perinatal morbidity and mortality. Despite significant recent efforts to define biomarkers to improve the ability of clinicians to predict, diagnose and manage preeclampsia, none perform well enough to have broad clinical utility. Since preeclampsia is known to affect lipid metabolic pathways, we set out to validate and quantitate the plasma lipidome from women with severe preeclampsia (sPE) and preterm labor (PTL) using the Lipidizer™ Platform (SCIEX). Lipid profile abnormalities were found in the triacylglycerides species (TAGs) and diacylglycerides species (DAGs) as well as some novel markers found to increase in Preeclampsia. Plasma from women with sPE (gestational age 25-37 weeks) were analyzed. Gestational age-matched plasma samples from women with PTL were used as controls. Applying internal standards (Avanti Polar Lipids), plasma was extracted and the Lipidizer™ Platform (SCIEX) was used for targeted profiling of approximately 1100 lipid molecular species from 13 different lipid classes. This strategy allowed for i) quantitative results for each lipid class as a sum of individual species ii) mole percent composition obtained computationally from lipid molecular species data and iii) accurate lipid species compositions. Coefficients of variation for all species were  $<6\%$ , highlighting excellent reproducibility of the assay. Plasma from women with severe preeclampsia primarily showed differences in the TAG and DAG species as compared with preterm labor controls. The data were probed for pathway interactions and statistical analyses and a novel set of lipid molecular species were found to increase significantly. A subset of these markers which increase and the TAG and DAG molecular species which decreased could be used to validate these potential markers at earlier gestational ages to better predict, diagnose and manage preeclampsia.

**Poster #: 459**

**Abstract #: 2495**

**Abstract Title:** Identification of CSF metabolite panel for simultaneously differentiating different types of autoimmune diseases of CNS

**Authors:** SOOJIN PARK,

**Presenting Author Affiliation:** Kookmin university

**Abstract Submission:**

Auto-immune inflammatory disease of central nervous system (CNS) occurs when immune systems, which help protect against foreign substances such as viruses and bacteria, attack and destroy healthy body tissues. Inflammatory demyelinating disease of CNS includes multiple sclerosis (MS), neuromyelitis optica spectrum disease (NMOSD), and myelitis (MY). The diagnosis based on a combination of clinical criteria, MRI, and neurophysiology could be a lengthy and expensive process thus, the discovery of single diagnostic and prognostic markers is critical.

Metabolomics is the systematic study of identifying and quantifying small molecule, which is often ideal strategy for tracking molecular dynamicity triggered by abnormal status (e.g. disease).

In this study, we investigated the unique metabolic features of different type of auto-immune disorders, and discovered robust biomarker panel for clinical application. High-throughput and non-targeted metabolite profiling was performed on 145 CSF, which included 12 control, 54 MS, 49 NMOSD, and 30 MY.

The primary statistical analysis determined significant biochemical modulation including atypical increases of 1-monopalmitin, 1-monostearin in all three autoimmune diseases compared to the control samples. Subsequently, we constructed the universal metabolite composite which simultaneously discriminated each disease from other groups, and generated multi-factor interactive disease metabolic networks, which may help mechanistic understanding of pathophysiology reflected in CSF. Further, we revealed transition status-specific metabolic regulation where glycolic acid and capric acid showed gradual increases along with the stage transition (remission-relapse).

**Poster #: 460**

**Abstract #: 2198**

**Abstract Title:** LC-HRMS reveals distinct circulatory metabolic profiles of asthma severity

**Authors:** Stacey Reinke, Héctor Gallart-Ayala, Antonio Checa, Alexander Fauland, Cristina Gómez, Shama Naz, Ratko Djukanovic, Timothy Hinks, Craig Wheelock,

**Presenting Author Affiliation:** Karolinska Institutet

**Abstract Submission:**

Asthma is a heterogeneous pulmonary disease, characterized by chronic airway inflammation. Disease etiology is multifactorial and not fully elucidated. Clinical presentation is diverse, ranging from mild disease, which is well controlled by intermittent treatment, to severe disease, which is poorly controlled and differentially responsive to long-term treatment regimes. We hypothesized that LC-HRMS based metabolomics would provide insight to underlying disease mechanisms, as they relate to disease severity.

Serum was collected from healthy controls (N=22) and mild (N=12), moderate (N=20), and severe (N=24) asthmatic individuals, classified a priori by clinical measures. Protein-free extracts were analyzed using LC-HRMS employing reversed-phase and HILIC chromatography coupled to a Q-Exactive Orbitrap mass spectrometer using ESI. Sequence order was randomized and pooled quality control (QC) samples were injected throughout the sequence. Metabolites were matched to accurate mass and retention time (AMRT) and confirmed by MS/MS using an in-house chemical reference library. Prior to data analysis, signal correction and data cleaning were performed metabolites with an RSD >25% or >20% missing values were excluded. Metabolites of interest were confirmed using targeted MS-based methods.

Sixty-six metabolites were included in analyses fifteen were significantly altered ( $p < 0.05$ ). Four metabolites (oleoylethanolamide, 22-hydroxycholesterol,  $\alpha$ -linolenic acid, sphingosine-1-phosphate) increased in asthma, 4 decreased (DHEA-S, cortisone, pipecolate, cortisol) and 7 differentially changed with respect to severity (methylthioadenosine, prolylhydroxyproline, N-palmitoyltaurine, phenylalanine, xanthine, arginine, taurine). DHEA-S, cortisone, pipecolate, cortisol, prolylhydroxyproline, and N-palmitoyltaurine were also significantly correlated with inhaled corticosteroid dose. Multivariate analysis revealed a significant mean separation between severe asthmatics and all other clinical groups and a mean separation between mild asthmatics and healthy controls. Together, our findings show that (i) there is a modest underlying metabolic shift associated with asthma in circulation, (ii) this shift is distinct with respect to disease severity and involves diverse metabolic pathways, and (iii) steroid treatment significantly affects metabolism.

**Poster #: 461**

**Abstract #: 2391**

**Abstract Title:** An Integrated Workflow for Qualitative Flux Analysis by Accurate Mass LC/MS

**Authors:** Norton Kitagawa, Alex Apffel, Crystal Cody, Justin Cross, Ed Darland, Xinning Jiang, Don Li, Steve Madden, Yinghang Yang,

**Presenting Author Affiliation:** Agilent Technologies, Inc.

**Abstract Submission:**

Clinical Research

The field of metabolic flux analysis suffers from data analysis challenges. Creation of useful quantitative models is very difficult and can take even expert users a very long time to complete. Much of the data mining of stable isotope labeled compounds involves either difficult-to-use software or manual processing with user-created spreadsheets. Once results are generated, it is difficult to visualize them on pathways and communicate these results to collaborators and scientific journals.

A target list containing TCA pathway metabolites were constructed from a metabolite database. Chondrosarcoma cells with a known isocitrate dehydrogenase (IDH2) mutation were incubated with U-13C-Glutamine and U-13C-Glutamate and extracted at various time points after label introduction: 12C (unlabeled), 0.5 hr, 1 hr, 2 hr, 3 hr and 8 hr. LC/MS analysis was performed using ion pair C18 reverse phase chromatography on an accurate mass LC/MS using negative ion acquisition operating in high resolution mode.

Raw data was automatically feature extracted for potentially label incorporation into target pathway metabolites. The feature extraction program enabled fast review, editing, quality metrics and dedicated isotopologue data visualizations. All isotopologue mining results were visualized on BioCyc glycolysis and TCA cycle pathways in a pathway visualization. Time-dependent label incorporation of 13C atoms could be observed throughout the TCA cycle in several monitored metabolites, as well as an overall reduction in metabolite pools for specific TCA metabolites.

For research use only. Not for use in diagnostic procedures.

**Poster #: 462**

**Abstract #: 2376**

**Abstract Title:**  $^1\text{H}$ -NMR-based metabolomic analysis of urine from neonates with late-onset sepsis

**Authors:** Anastasia Chrysovalantou Chatziioannou, Kosmas Sarafidis, Agathi Thomaidou, Helen Gika, Emmanuel Mikros, Dimitra Benaki, Elisavet Diamanti, Vasiliki Drossou, Georgios Theodoridis,

**Presenting Author Affiliation:** School of Chemistry, A.U.Th., Greece

**Abstract Submission:**

Late-onset sepsis (LOS) is a major cause of increased morbidity and mortality especially in preterm neonates. To date, most biomarkers evaluated in LOS lack high diagnostic accuracy, a fact that hinders early and prompt initiation of treatment. The aim of this study was to evaluate the metabolic profile of neonates with LOS using proton nuclear magnetic resonance spectroscopy ( $^1\text{H}$ -NMR) and determine the possible value of urine metabolomics as an early diagnostic tool in LOS.

Prospective, case-control study evaluating urine samples collected at the time of initial diagnosis from 7 neonates with possible and 11 ones with confirmed LOS (all due to Gram-bacteria) as well as from 18 neonates without sepsis (controls). Moreover, urine was collected from each subject at three different time points (day 0, 3 and 10 of sepsis/controls evaluation). Urine metabolites were assessed using  $^1\text{H}$ -NMR analysis. Data pre-processing was performed with MATLAB software while statistical analysis was implemented using multivariate statistical software (SIMCA 13).

Multivariate statistical analysis and ANOVA documented significant variability in the metabolic profile of septic neonates vs. controls. PCA and OPLS-DA models showed clear separation between the studied cohorts. S-line plot analysis led to the identification of significant metabolites. Permutation plots validated the significance of all statistical models and monitoring of different time points of the disease verified suggested biomarkers. Metabolites enabling discrimination septic neonates vs. controls showed a clear recovery trend from disease to healthy condition over time.

In summary, neonates with possible and confirmed LOS have different metabolic profile from those without sepsis, allowing their discrimination with the use of  $^1\text{H}$  NMR-based urine metabolomic analysis. Findings of this study provide a clear evidence for the possible use of urine NMR-based metabolomics as an early diagnostic tool in neonates of ICUs with LOS.

**Poster #: 463**

**Abstract #: 2580**

**Abstract Title:** Biomarkers of nuts exposure: diet and host-microbial interaction in people with cardiometabolic risk

**Authors:** Cristina Andres-Lacueva,

**Presenting Author Affiliation:** Biomarkers and Nutrimetabolomics Laboratory, Depar

**Abstract Submission:**

S. Tulipani<sup>1</sup>, M. García-Aloy<sup>1</sup>, X. Mora-Cubillos<sup>1</sup>, M. Rabassa-Bonet<sup>1</sup>, M. Bulló<sup>2</sup>, L. Ferrucci<sup>3</sup>, S. Bandinelli<sup>4</sup>, A. Cherubin<sup>5</sup> and C. Andres-Lacueva<sup>1\*</sup>

<sup>1</sup>University of Barcelona, Biomarkers & Nutrimetabolomic Lab., Nutrition and Food Science Department, 08028, Spain <sup>2</sup>Universitat Rovira I Virgili, Human Nutrition Unit, IISPV, 43201, Spain. Instituto de Salud Carlos III (ISCIII), CIBER Fisiopatología de la Obesidad y Nutrición (CIBERObn), 15706, Spain, <sup>3</sup>Clinical Research Branch, National Institute on Aging, National Institutes of Health, 21225, USA, <sup>4</sup>Azienda Sanitaria Firenze, Geriatric Rehabilitation Unit, 50122, Italy, <sup>5</sup>Italian National Research Centre on Aging, Geriatrics and Emergency Care, 60124, Italy \*candres@ub.edu

Tree nuts have a recognized role in the improvement of components of the metabolic syndrome (MetS), but the mechanisms implied are not fully characterized. Part of these effects are recently ascribed to the metabolism of polyphenols highly abundant in nuts, which in turn necessarily require a functional diet and host-microbial interaction. Through a LC-ESI-qToF-MS-driven untargeted metabolomic approach, we first identified the most discriminant biomarkers of nuts exposure in 12-week mixed nuts intervention study (30 g/d) involving subjects with MetS. Urolithin A glucuronide, namely a product of the gut microbiota-host cometabolism of ellagitannins contained in walnuts, was the most discriminative dietary biomarker (nivel I identification ROC AUC=89.6% (80.8-98.4))] despite the inter-individual variation expected. Plasma levels of urolithin A glucuronide also showed a significant inverse correlation with abdominal adiposity (waist circumference:  $r=-0.550$  waist-hip ratio:  $r=-0.409$ ) and impaired glycemic control (FI:  $r=-0.414$  HOMA-IR:  $r=-0.417$ ) of the subjects at baseline, while positively associated with the reduction of adiposity following nuts intake. These data suggested the presence of a more urolithin prone-to-produce microbiota, and therefore a higher exposure to bioactive metabolites, in subjects with less severe MetS traits known to be associated with gut microbial dysbiosis. The generated hypothesis were verified by independently applying a similar metabolomics approach to a subcohort of the

**Poster #: 464**

**Abstract #: 2129**

**Abstract Title:** The effects of changing the liquid/solid content of an isoenergetic test meal on gastric emptying and lipidomics

**Authors:** Xuefei Li, Evelina Charidemou, Elise Orford, Sara Wassell, Matthew Harvey, Albert Koulman, Michelle Venables, Jules Griffin,

**Presenting Author Affiliation:** MRC Human Nutrition Research

**Abstract Submission:**

Gastric emptying (GE) is a complex physiological response to the presence of food, which is regulated by different factors including hormones, nervous system feedback control, and the mechanical and chemical properties of the meals ingested. Altered gastric emptying is associated with various diseases including diabetes, insulin resistance, and obesity. Adjusting meal composition and feeding frequency to mediate GE is a potential strategy to regulate appetite and food intake to ameliorate these disease states.

The aim of this study is to investigate whether the liquid, carbohydrate rich, component of a mixed meal delays gastric emptying of the solid portion of a mixed meal, impacts nutrient absorption, and induces changes in the plasma lipidome using stable isotopic labelling technique.

Twelve healthy male participants were recruited into a randomised, 5-way crossover study using 5 isoenergetic mixed meals (2MJ) with varying liquid/solid component ratios. The 5 meals contained either a typical UK macronutrient composition (Standard with or without bread) or high in fat, high in carbohydrate or high in protein. The standard meal with bread contains a solid form of carbohydrate provided by bread, and the other four meals contain a liquid form of carbohydrate provided by orange juice.

The <sup>13</sup>C octanoate breath test was used to assess GE. After fasting overnight, participants received one of the five meals randomly, each meal labelled with 100 µl [1-<sup>13</sup>C] octanoic acid. Breath and plasma samples were collected at various intervals for 6 hours. Isotopic enrichment of <sup>13</sup>C in breath samples were analysed by continuous flow isotope ratio mass spectrometry. Blood plasma were examined by LC-MS based lipidomics.

Bayesian hierarchical analysis will be performed for the breath samples to estimate GE parameters using the WinBUGS program. Multivariate statistics including principal components analysis and tools based on partial least squares will be used for the lipidomic analysis.

**Poster #: 465**

**Abstract #: 2282**

**Abstract Title:** Metabolomic exploration of the combined effects of perinatal exposure to bisphenol A and high fat diet on hepatic rats metabolism

**Authors:** Cecile Canlet, Marie Tremblay-Franco, Emilien Jamin, Roselyne Gautier, Jean-François Martin, Nicolas Cabaton, Florence Blas-Y-Estrada, Jeremie David, Sergio Polakof, Laurent Debrauwer, Daniel Zalko,

**Presenting Author Affiliation:** Toxalim, Université de Toulouse, INRA

**Abstract Submission:**

Over the last few years, many studies have demonstrated that a perinatal exposure to estrogenic endocrine disruptors, can impact the offspring. Bisphenol A (BPA), a chemical used worldwide to manufacture polycarbonate plastics and epoxy resins, is a model molecule in many of these studies. In addition to its effects on reproduction, exposure of pregnant rodents to low doses of BPA has been postulated to be a contributing factor in predisposing populations to the development of obesity and diabetes later in life, in the same way as a high fat diet.

In this context, this work aims at studying in rats the impact of a perinatal exposure to low doses of BPA in combination with a high-fat diet, using a global metabolomics approach based on both proton NMR- and HRMS-based metabolomics.

Experimental design: pregnant rats were exposed to low doses of BPA (0.05 and 5 µg/day/kg) in combination with a normal fat diet (NF) or a high-fat diet (HF) during gestation and lactation. After PND21 (weaning), male animals from the F1 generation were either fed the NF or the HF diet, until PND142. Male livers extracts from PND21 and PND142 were prepared and submitted to proton NMR spectroscopy, and LC-HRMS analyses.

Multivariate statistical analyses of PND21 liver NMR and HRMS data allowed to significantly discriminate the different groups according to the diet (HF and NF). Conversely, no difference was observed between control animals and animals exposed to BPA using NMR spectroscopy, while control and exposed animals groups can be separated using HRMS.

For PND142 pups with a similar perinatal diet scenario, control animals and animals exposed to 5 µg/day/kg BPA displayed different metabolic fingerprints. Metabolites differentially modulated have been identified using 2D NMR (twenty metabolites) and MS/MS (fifty metabolites) experiments. These metabolites will subsequently be used for metabolic network reconstruction.



**Poster #: 466**

**Abstract #: 2426**

**Abstract Title:** Using metabolomics and proteomics to identify novel biomarkers of wood quality in Mediterranean pine (*Pinus pinaster* Aiton)

**Authors:** Mónica Meijón, Isabel Feito, Juan Majada, Luis Valledor,

**Presenting Author Affiliation:** University of Oviedo

**Abstract Submission:**

Natural variation of the metabolome and proteome of *Pinus pinaster* was studied to improve our understanding of environmental adaptation processes, phenotypic diversity, and wood quality. The metabolomes and proteome of needles and the apical and basal section of buds were analyzed in three provenances of *P. pinaster* with contrasting growth capacity and forest qualities selected from mountain in the northwest (CDVO) to the coastal region of southeast Spain (ORIA) also considering a provenance from a very sandy Moroccan area (TAMR). The three provenances were grown in a common garden for five years and metabolite and protein extraction were performed through Valledor et al. (2014) protocol. For metabolite detection two complementary mass spectrometry techniques: GC-MS and LC-Orbitrap-MS were used to reach a higher coverage of metabolome while for protein identification GeLC-Orbitrap/MS combined with the development of custom peptide databases was used. Metabolome, proteome and environmental and growth data were integrated employing modelling and statistical tools to provide a comprehensive picture of phenotypic diversity.

A total of 1590 metabolites and 1453 proteins were identified. The analysis of the metabolome showed that differences were maintained across provenances and that the metabolites characteristic of each developmental stage are related to primary metabolism, while provenances were distinguishable when developmental stages were analyzed independently. Integrative analyses of metabolome, proteome and environmental data showed three population clusters, in relation to aridity conditions of origin and wood quality, being secondary metabolites, and in particular flavonoid and terpenoid pathways, essential to reach this differential clustering. Additionally, some key enzymes were linked by sPLS networks to wood quality. Altogether these results provide a new perspective of how tree metabolism adapt to different environment, and how these adaptations are also reflected in wood quality, providing these results a new set of biomarkers for breeding programs and forest management practices.

**Poster #: 467**

**Abstract #: 2582**

**Abstract Title:** Untargeted Urine Metabolome Profiling of Infants from the Danish SKOT I and SKOT II Cohorts

**Authors:** ceyda pekmez, Lena Kirchner Brahe, Anni Larnkjær, Christian Mølgaard, Kim Michaelsen, Lars Dragsted,

**Presenting Author Affiliation:** Department of Nutrition, Exercise and Sports, Univ

**Abstract Submission:**

**Aim:** There is a growing evidence that the mother's nutritional status in pregnancy affects metabolism of the offspring. However, the link between mother's nutritional status in pregnancy and infant's metabolism is still not clear. The aim of this study is to explore whether the urine metabolome profile is different between infants born from obese mothers and mothers with body mass index (BMI) within the normal range.

**Method:** From the Danish SKOT 1 cohort of normal-weight mothers and children, a randomly selected set of 30 term infants were selected and compared with 40 term infants from SKOT 2 cohort of obese mothers. Urine samples collected with cotton inserts in the diapers or at the age of 9 months and samples collected by normal spot voids at 36 months were included the study. Urine samples were diluted and profiled using UPLC-Q-TOF/MS. Data were preprocessed with MZmine\_2.19 followed by principal component analyses (PCA) and Partial Least Squares Discriminant Analysis (PLSDA) using PLS\_Toolbox\_8.1.

**Results:** There was a clear difference in urine metabolome profiles by PCA at the age of 9 and 36 months. At 9 months samples from SKOT 1 and 2 were clearly separated by PCA based on urine metabolome profile, but for the age of 36 months there was only a tendency for separation in PCA. Late eluting, fat soluble compounds with high VIP scores –possibly related to mother's BMI- were detected in the PLSDA model at 9th and 36th months.

**Conclusion:** Further analyses are needed to identify the compounds related to mother's BMI and interpret the effect observed on offspring metabolism.

**Poster #: 468**

**Abstract #: 2205**

**Abstract Title:** New databases on food compounds and the food metabolome to support discovery of novel dietary biomarkers – A FoodBALL initiative

**Authors:** Claudine Manach, Christoph Weinert, Lars Ove Dragsted, Stéphanie Durand, Franck Giacomoni, Craig Knox, Sabine Kulling, Rafael Llorach, Vanessa Neveu, Giulia Pratico, Estelle Pujos-Guillot, Albert Remus Rosana, Joe A. Rothwell, Ta

**Presenting Author Affiliation:** INRA

**Abstract Submission:**

One of the aims of the FoodBALL (Food Biomarkers Alliance) project (<http://foodmetabolome.org>) is to develop the missing tools and resources to facilitate the identification of food intake biomarkers using metabolomics. A compound database with extensive coverage of the food metabolome and a food intake biomarkers database were identified as priority needs. The most comprehensive database on food constituents and their chemical and biological properties, FooDB ([www.foodb.ca](http://www.foodb.ca)), as well as the expert-curated database PhytoHub ([www.phytohub.eu](http://www.phytohub.eu)) dedicated to dietary phytochemicals are being enriched to include new data on food non-nutrients and their human metabolites, including the known metabolites described in the literature and in silico predicted metabolites. In parallel, a new database, Exposome-Explorer, is being developed to include all known dietary biomarkers (currently n=142) and rich information on their measurement in various populations.

Beyond databases, the lack of commercial chemical standards for food-derived metabolites is another major limitation for the annotation of unknown signals in nutritional metabolomic studies. FoodComEx (Food Compound Exchange, <http://foodcomex.org/>) is a new chemical library aiming at facilitating the sharing of not easily accessible standards for diet-related compounds. FoodComEx is an online catalog of pure compounds made available by academic laboratories. Compounds are stored in the laboratory where they have been isolated or synthesized. Anyone interested in one compound can contact the provider and a bilateral negotiation will define the terms of collaboration, within the rules defined in a charter of good practices. FoodComEx is a collaborative initiative widely open to new contributors and users.

Another resource developed in the FoodBALL project is a web portal (<http://foodmetabolome.org/wpkg4>) which presents and links to the most useful tools, databases, libraries of spectra, and softwares for nutritional metabolomics and dietary biomarker discovery. It will also propose tutorials, webinars, and news related to the Food metabolome.

Funding: JPI HDHL FoodBALL project (2014-2017)

**Poster #: 469**

**Abstract #: 2224**

**Abstract Title:** Resting energy expenditure is not associated with distinct plasma and urine metabolite profiles in healthy humans

**Authors:** Achim Bub, Manuel Armbruster, Manuela Rist, Lara Frommherz, Christoph Weinert, Diana Bunzel, Carina Mack, Björn Egert, Benedikt Merz, Alexander Roth, Sabine Kulling, Bernhard Watzl,

**Presenting Author Affiliation:** Max Rubner-Institut

**Abstract Submission:**

Lean body mass (LBM) substantially impacts human metabolism and is a major determinant of resting energy expenditure (REE). Differences in REE between men and women mainly result from sex related differences in LBM. So far, little is known if REE and LBM are reflected by a distinct human metabolite profile. Therefore, we aimed to identify plasma and urine metabolite patterns that are associated with REE and LBM of healthy men and women.

We investigated 301 healthy male and female subjects (18 – 80 years) under standardized conditions in the cross-sectional KarMeN study (Karlsruhe Metabolomics and Nutrition). REE was determined by indirect calorimetry and LBM by dual x-ray absorptiometry. Fasted blood and 24h urine samples were analyzed by targeted and untargeted metabolomics methods using GC×GC-MS, GC-MS, LC-MS and NMR. Data were evaluated by predictive modelling of combined data using different machine learning algorithms, namely SVM, glmnet and PLS.

For the participants of the KarMeN study LBM correlates with REE ( $r = 0.877$  linear regression). However, the applied machine learning algorithms did not reveal a metabolite profile predictive for REE or LBM, when analyzing data for men and women, separately. When evaluating data of men and women combined, as it has been described by others, we were able to predict REE and LBM with high accuracy (>90%). This, however, was a clear effect of sex, which is supported by the high degree of overlap in identified important metabolites for LBM, REE and sex, respectively. We conclude that studies in healthy humans applying metabolomics need to consider sex specific data evaluation.

**Poster #: 470**

**Abstract #: 2267**

**Abstract Title:** Stressed? Metabolomics can help. A metabolomics comparison of two rat models for stress.

**Authors:** Karl Fraser,

**Presenting Author Affiliation:** AgResearch

**Abstract Submission:**

It is well known that our gut health can affect our physical health and there is accumulating evidence pointing strongly to a role for gut microbiota also manipulating our mental health. Yet, just how this interaction occurs between our brain and the gut microbes remains largely unknown. What is clear is that biochemical signalling along the “gut-brain-axis” is a bi-directional process. In our study, we examined the effects of stress and anxiety behavioural tests on the plasma and brain metabolome in Sprague-Dawley (SD) and Wistar Kyoto (WKY) rats. While SD rats are a routinely used model for ‘normal’ physiological states, the WKY rat strain demonstrates a hyper-responsiveness to stress and is commonly used to study gastrointestinal disorders. Half the SD and WKY rats were subjected to the Open Field and Forced Swim Tests, while the remaining half remained behavioural testing naive. Plasma and brain extracts were analysed with both positive and negative ionisation modes of lipid and HILIC (polar compounds) liquid chromatography mass spectrometry (LC-MS) streams. As expected, the rat strains could be clearly distinguished within both the plasma and brain metabolomes. However, differential features/compounds of stress were also observed between behaviourally tested and untested rats of the same strain in both plasma and brain samples. Key differential metabolites in the plasma included those related to glutamine, glutamate, and dopamine pathways, pathways known to be involved in neurotransmission. Decreased sphingomyelin levels were observed in the brain extracts within the WKY rat brain when subjected to stress. Behavioural challenges were also associated with changes in the gastrointestinal microbiome. This study demonstrates that metabolomics is capable of detecting differences in both plasma and brain samples from two rat models exposed to stress, providing further evidence that physiological, behavioural, and microbiome responses are intimately linked and can be specific to each rat strain.

**Poster #: 471**

**Abstract #: 2316**

**Abstract Title:** Metabolomics Reveals Tomato Alkaloids as Novel Markers of Tomato Consumption

**Authors:** Morgan Cichon, Ken Riedl, Steven Clinton, Steven Schwartz,

**Presenting Author Affiliation:** The Ohio State University

**Abstract Submission:**

Diets rich in tomatoes have been associated with a decreased risk of chronic diseases. However, the mechanism behind this observed protective effect remains unclear. Much research has focused on the tomato carotenoid lycopene, but tomatoes contain many other potentially bioactive phytochemicals. The objective of this work was to compare the effects of lycopene and tomatoes on the metabolomes of mice. Male C57BL/6 mice (10/group) were fed an AIN-93G control diet or the same diet supplemented with red tomato powder or lycopene for 4 weeks. Untargeted metabolomic profiling of plasma and liver was performed using UHPLC-QTOF-MS and resulting data were processed and analyzed using Agilent Profinder and Mass Profiler Professional software. Of the thousands of plasma metabolites detected using this approach, 169 were found to be significantly different ( $P < 0.05$ ) between the red tomato, lycopene, and control fed mice. Differentiating plasma metabolites include both endogenous as well as exogenous compounds from the diets. Most notably, several tomato steroidal alkaloids were detected in plasma after the consumption of tomatoes. One of these metabolites has been positively identified as the tomato alkaloid tomatidine and another has been tentatively identified as the tomato alkaloid pimpifolidine. It appears that glycoalkaloids present in the tomato diet are cleaved in the gastrointestinal tract to the observed aglycones. Retention times and mass spectral information of other diet derived plasma metabolites are suggestive of additional steroidal alkaloids and their hydroxylated/reduced metabolites. To our knowledge, tomato steroidal alkaloids have not been reported in plasma previously and may contribute to some of the health promoting properties associated with tomatoes. Work is being conducted to determine if these results translate to humans.

**Poster #: 472****Abstract #: 2474**

**Abstract Title:** Consumption of rye and wheat bran fractions increases levels of novel betaines in metabolically active tissues in a mouse model

**Authors:** Jenna Pekkinen, Olli Kärkkäinen, Ville Koistinen, Natalia Rosa-Sibakov, Jukka Leppänen, Marko Lehtonen, Valerie Micard, Kaisa Poutanen, Hannu Mykkänen, Kati Hanhineva,

**Presenting Author Affiliation:** University of Eastern Finland

**Abstract Submission:**

Besides being an important source of dietary fiber, whole grains contain a plethora of bioactive phytochemical species such as phenolic acids, lignans, alkylresorcinols, and benzoxazinoids that supposedly possess various health protective effects once harbored human body via diet. Additionally, whole grains are one of the richest dietary sources of glycine betaine, a trimethylated derivative of amino acid glycine, that is required not only for maintaining normal cellular physiology via regulating osmotic balance but also as a methyl donor in several important biochemical reactions maintaining metabolic health. In our recent investigation on a mouse dietary model fed with various rye and wheat bran fractions a group of novel betainized compounds not reported in mammals earlier were found to be excreted in urine. These included various di- and trimethylated amine species such as several amino acid derived betaines, pipelicolic acid betaine and trigonelline. Several of the betainized compounds were also present in the fasting plasma and in several tissues of the mice, although in much lower levels than glycine betaine. On contrary, one of the betainized compounds was clearly accumulated in metabolically active tissues including heart, muscle, pancreas, brown adipose tissue, and liver with similar or even higher intensities than glycine betaine. We have located the presence of this novel betainized compound also in other mammalian tissues, including pig and human heart, where it potentially contributes to such metabolic processes that may be related to the health protective effect of whole grain rich diets. Investigation of the bran products used in the mouse feeding revealed, that the compounds were only marginally present or completely absent from the diet. Based on our analysis of intestinal tissue samples these novel betainized compounds are most likely originated from the gut as metabolites of intestinal microbiota.

**Poster #: 473**

**Abstract #: 2504**

**Abstract Title:** Metabolomic analysis of Fusarium head blight on wheat – elucidation of fungal attack

**Authors:** Alexandra Simader, Alexandra Parich, Manuel Hofer, Michael Sulyok, Maria Doppler, Christoph Bueschl, Barbara Steiner, Marc Lemmens, Hermann Buerstmayr, Gerhard Adam, Justyna Rechthaler, Rudolf Krska, Rainer Schuhmacher,

**Presenting Author Affiliation:** BOKU (Vienna), IFA-Tulln

### **Abstract Submission:**

#### Introduction

The plant pathogen *Fusarium graminearum* (Fg) is able to infect wheat plants thereby leading to severe yield and quality losses as well as contamination with mycotoxins. In order to develop knowledge-based strategies against Fusarium head blight (FHB) it is greatly desired to systematically investigate the interactions between Fg and wheat plants.

#### Methods

In a recently performed metabolomics experiment two near isogenic wheat lines differing in resistance QTLs *Fhb1* and *Qfhs.ifa-5A* were inoculated in the glass house with Fg with the aim to investigate changes in the metabolome over time. Resistant CM-NIL-38 and the susceptible wheat line CM-NIL-51 were harvested at 0, 3, 6, 12, 24, 36, 48, 72, 96 hours after treatment with Fg. Mock and no treatment served as control. The use of a <sup>13</sup>C-assisted untargeted LC-HRMS approach allowed to investigate the secondary metabolites, whereas additional targeted GC-MS analysis was applied to complement the data by primary metabolites. Moreover, Fg reads from the transcriptomic analysis of the same data set served as reference for the fungal biomass. In order to discriminate between fungal attack and defense response of the host plant, metabolomics time course data such as Fg secondary metabolites as well as putative fungal biomarker compounds (e.g. mannitol, arabitol, xylitol, threitol, chitin, stachydrin) were investigated.

#### Results

Fg secondary metabolites and putative fungal biomarkers were detected as early as 24-36 hours after inoculation (hai). Time course of their formation has been investigated in detail and revealed mostly higher abundances of Fg secondary metabolites in the susceptible genotype at later time points, whereas at earlier time points some fungal metabolites were more abundant in the resistant wheat line. Additionally some metabolites could be investigated solely in Fg-treated samples exhibiting similar time course in the resistant genotype compared to the susceptible wheat line.



**Poster #: 474**

**Abstract #: 2505**

**Abstract Title:** Multi-compartmental metabolomics study of a C56BL/6J diet-induced obesity mouse model

**Authors:** Christian Yde, Jette Young, Lotta Stenman, Kyle Glover, Henrik Max Jensen,

**Presenting Author Affiliation:** Aarhus University

**Abstract Submission:**

The worldwide obesity epidemic and changes in lifestyle to less physical activity have given rise to growing numbers of people suffering from metabolic syndrome. The aim of the present study was to measure the effect of consumption of a high-fat diet in comparison with a low-fat diet, because this is one of the key factors in developing metabolic syndrome. To elucidate the underlying molecular mechanism, metabolomics analysis was performed. C57BL/6J mice that were fed a high-fat diet (60% fat) for 18 weeks developed traits associated with metabolic syndrome such as weight gain, reduced glucose tolerance and altered serum biomarkers. <sup>1</sup>H NMR metabolic profiling was used to analyze urine, plasma, intact liver and dual-phase extractions of liver and adipose tissue. The strongest effect of the two treatments was found for the water-MeOH extractions of liver and adipose tissue. Metabolic changes were associated with changes in energy, amino acid and microbial metabolism. The high-fat diet induced higher creatine turnover, hepatic lipid and taurine levels and decreased trimethylamine excretion as well as strong biomarkers in the liver. Furthermore, the NMR data were integrated with GCMS/LCMS data, and correlations with metadata were performed. In conclusion, the metabolic changes caused by consumption of a high-fat diet can be used as a metabolic profile of the metabolic syndrome related to obesity.

**Poster #: 475**

**Abstract #: 2515**

**Abstract Title:** Targeted metabolomics reveals extended postprandial effects of whole grain rye porridges fortified with inulin and gluten and suggests potential mechanism of second meal effect

**Authors:** LIN SHI, Carl Brunius, huaxing Wu, Isabella Lee, Rikard Landberg, Ali Moazzami,

**Presenting Author Affiliation:** Food Science Department, Swedish University of Agr

**Abstract Submission:**

Intervention studies have shown beneficial effects of whole-grain (WG) rye foods on appetite, postprandial glucose, and insulin sensitivity, which may be of relevance for prevention of chronic metabolic diseases and their pre-conditions. In a recent study, we observed that consumption of different WG rye porridge breakfasts increased satiety and reduced blood glucose after lunch compared with refined wheat bread. This study has investigated postprandial metabolic changes after interventions in the same study material, with the aim to explore mechanisms for the observed effects.

Healthy subjects (11 men and 10 women) consumed six isocaloric breakfasts in a randomized, cross-over design. Test breakfasts were: two rye porridges (40 g and 55 g WG rye), three rye porridges with addition of inulin and gluten (40 g WG rye + inulin and gluten in a ratio of 9:3 g 6:6 g 3:9 g) and a wheat bread control (55 g refined wheat bread). A standardized lunch was served 4h after breakfasts. Plasma metabolic profile was assessed by targeted NMR metabolomics and short chain fatty acids (acetate, propionic and butyric acid) were measured by GC-MS from baseline and regularly during 8h.

Time-dependent alterations between breakfasts were observed in 36 postprandial metabolites. Plasma amino acids concentrations reflected composition in the breakfasts, with disproportionally higher concentrations from gluten fortification, but no associations were observed with appetite and insulin response. Rye porridges resulted in lower plasma glucose and higher short chain fatty acid concentrations after lunch compared with refined wheat bread, in an inulin dose-dependent manner. Breath hydrogen was positively correlated with acetate and negatively with glucose concentrations.

Postprandial metabolic alterations between rye porridges and refined wheat bread suggest that colonic fermentation may play an important role in attenuating plasma glucose response after subsequent meal. However, such associations were not reflected effects on appetite.

**Poster #: 476**

**Abstract #: 2187**

**Abstract Title:** Host: microbiome co-metabolic processing of dietary polyphenols – an acute, single blinded, cross-over study with different doses of apple polyphenols in healthy subjects

**Authors:** Marynka Ulaszewska, Kajetan Trost, Jan Stanstrup, Davide Albanese, Carlotta De Filippo, Kieran Tuohy, Fausta Natella, Cristina Scaccini, Fulvio Mattivi,

**Presenting Author Affiliation:** Fondazione Edmund Mach, Food Quality and Nutrition

**Abstract Submission:**

Apples are one of the most commonly consumed fruits and yet we still do not fully understand how they are metabolized by the human body. Here we studied the nutrikinetics of apple polyphenols in cloudy apple juice and polyphenol-enriched apple juice using LC-HRMS based metabolite profiling. Healthy volunteers participated in an acute single blind controlled crossover study in which they consumed 250 mL of cloudy apple juice (Crispy Pink apple variety), or 250 mL of the same juice enriched with an apple polyphenol extract (750mg). Plasma, and urine were collected at time 0, 1h, 2h, 3h, 5h, together with a 24h urine sample. Faecal samples were collected from each individual during the study for 16S rRNA gene profiling. 110 metabolites were significantly elevated following intake of polyphenol enriched cloudy apple juice. Single intake of the enriched apple juice did not significantly alter the gut microbiota composition but faecal bacteria were correlated with specific microbial metabolites derived from apple polyphenols. Human metabolism of apple polyphenols is a co-metabolic process between human encoded activities and those of our resident microbiota. Here we have identified specific blood and urine metabolic biomarkers of apple polyphenol intake and identified putative associations with specific genera of gut bacteria, associations which now need confirmation in specifically designed mechanistic studies.

**Poster #: 477**

**Abstract #: 2418**

**Abstract Title:**  $^{13}\text{C}$  and  $^{15}\text{N}$  Assisted Metabolite Profiling of the Plant Pathogen *Fusarium graminearum* for the Study of its Interaction with Wheat

**Authors:** Bernhard Wolf, Bernhard Kluger, Christoph Bueschl, Maria Doppler, Andrea Koutnik, Marc Lemmens, Romana Stücker, Gerhard Adam, Rudolf Krska, Rainer Schuhmacher,

**Presenting Author Affiliation:** BOKU University Vienna, IFA-Tulln, AZ

**Abstract Submission:**

Global metabolomics of plant-fungi interaction is still hampered by the difficulty to properly dissect the metabolomes of the interaction partners. In the present study we have used  $^{13}\text{C}$ - and  $^{15}\text{N}$ - stable isotope labelling (SIL) in combination with LC-HRMS to assign the metabolome of the plant pathogenic fungus *Fusarium graminearum* (Fg). Subsequently, our study aimed at the detection of the fungal metabolites in Fg treated wheat plants action partner.

To this end, fungal cultures of the *F. graminearum* wild type strain PH-1 were cultivated in parallel in minimal media containing glucose and sodium nitrate either in its native ( $^{12}\text{C}$  /  $^{14}\text{N}$ ) or U- $^{13}\text{C}$  /  $^{14}\text{N}$  and  $^{12}\text{C}$  / U- $^{15}\text{N}$  labeled media, both with a high degree of enrichment (> 99%) of the respective heavy isotope. Subsequent C18 LC-HRMS analysis and data processing by our in-house software MetExtract II revealed more than 600 truly Fg derived metabolic features which correspond to more than 150 feature groups, i.e. metabolites. For each detected metabolite ion the exact number of carbon and nitrogen atoms was determined based on both labeling approaches. This allowed to reduce the number of possible sum formulas and to annotate unknown metabolites. Next, metabolites, found in these fungal culture samples were used to create a positive list and screened for in wheat samples of a biological time course experiment. Non-treated, mock treated as well as Fg treated wheat samples were inspected for the presence of fungal metabolites.

Differential analysis of the tested plant samples revealed numerous metabolites, which have only been detected in Fg treated wheat and fungal cultures. The presented approach allowed to differentiate between fungal attack and plant defense processes and to investigate more details of fungal activities and metabolic pathways in planta.

**Poster #: 478**

**Abstract #: 2588**

**Abstract Title:** LC-MS profiling for discovery and identification of dietary exposure biomarkers in human urine

**Authors:** Thomas Wilson, Amanda Lloyd, Naomi Willis, Long Xie, Kathleen Taillart, Hassan Zubair, Edward Chambers, Gary Frost, John Mathers, Manfred Beckmann, John Draper,

**Presenting Author Affiliation:** IBERS

**Abstract Submission:**

An understanding of causal relations between diet and health is hindered by the lack of robust biological markers of food exposure. Most dietary biomarkers currently have been identified on the basis of knowledge of food composition by using hypothesis-driven approaches. However, the rapid development of metabolomics technology coupled with the availability of metabolite databases offers opportunity for the identification of novel biomarkers for the intake of a range of foods including fruit, vegetables, beverages, meats and complex diets. We are aiming to discover and validate potential urinary biomarkers in both controlled clinical and epidemiological contexts with a range of metabolomic techniques. Robust High Performance Computational (HPC) methods have been developed to pre-process and analyse LC-MS data of high complexity derived from ultra-high mass accuracy Orbitrap instruments. A non-targeted, data-driven approach using Flow Infusion-High Resolution Mass Spectrometry Fingerprinting followed by supervised multivariate classification and feature selection has been developed to identify putative dietary biomarkers in spot urine samples. In order to cover a range of metabolite chemistry and elucidate further potential biomarkers we employed high-throughput Ultra High Performance Liquid Chromatography-High Resolution MS, using both Reverse Phase C18 and Hydrophilic Interaction Chromatography. The combination of chromatography, accurate mass and tandem mass spectrometry means that we can accurately elucidate potential biomarkers after modelling, without the need for extensive targeted studies. Machine learning classification and feature selection supported by HPC routines provides robust validation of explanatory features in biomarker discovery studies. Potential food biomarkers from epidemiological studies are being validated by quantification in biofluid samples obtained from controlled clinical studies.

**Poster #: 479**

**Abstract #: 2104**

**Abstract Title:** High-throughput quantitation of metabolites and co-factors from the homocysteine-methionine cycle in plasma and cerebrospinal fluid

**Authors:** Seu Ping Guiraud, Laeticia DaSilva, Ivan Montoliu, Loïc Dayon, Antonio Núñez Galindo, John Corthésy, Martin Kussmann, François-Pierre Martin,

**Presenting Author Affiliation:** Nestlé Institute of Health Sciences SA

**Abstract Submission:**

The methionine cycle is a key regulatory node for human health with a clear link to cardiovascular disease and cognitive impairment, the latter ranging from mild cognitive decline to vascular dementia and Alzheimer's disease. Providing the nutritional co-factors for proper functioning of the methionine cycle may improve methylation, protect the brain from damage and reduce the risk of cardiovascular events. From a nutritional perspective we are genuinely interested in understanding these molecular processes at play. Today there is no single analytical method to monitor both metabolites and micronutrients at the same time across this biochemical pathway. To address this, we report here a new method for simultaneous quantitation of seventeen metabolites in the methionine cycle, i.e., homocysteic acid, taurine, serine, cysteine, glycine, homocysteine, riboflavin, methionine, pyridoxine, cystathionine, pyridoxamine, S-adenosylhomocysteine, S-adenosylmethionine, betaine, choline, dimethylglycine, and 5-methyltetrahydrofolic acid. This multiplexed method relies on ultra-performance liquid chromatography tandem mass spectrometry and was validated for the analysis of human plasma and cerebrospinal fluid samples. The assay provides highly accurate and precise quantitation of the 17 metabolites, whilst requiring only a sample volume of 50 µL. Moreover, the method is based on a simple sample preparation protocol, which - combined with a short chromatographic runtime - ensures a relatively high sample throughput. This analytical workflow thus provides a novel metabonomic approach for large-scale observational and interventional studies, in particular in humans. We expect our method to be critical for broad and deep molecular and nutritional phenotyping of individuals in relation to their dietary requirements, health maintenance and disease management.

**Poster #: 480**

**Abstract #: 2309**

**Abstract Title:** Metabolomics aids in discovery of putative bioactive compounds from tomatoes in a model of keratinocyte carcinoma of the skin

**Authors:** Jessica Cooperstone, Matthew Teegarden, Morgan Cichon, Kathleen Tober, Tatiana Oberyshyn, Steven Schwartz,

**Presenting Author Affiliation:** The Ohio State University

**Abstract Submission:**

Adding tomatoes to the diet has been shown to lessen erythema and inflammation after exposure to ultraviolet light in humans. Carotenoids, ubiquitous plant pigments responsible for the red color of tomatoes, have received the most attention for this photoprotective effect. However, single carotenoids appear less effective than whole foods suggesting other secondary plant metabolites may play a role. Our group has observed that tomato supplementation can decrease tumor number in a murine model of ultraviolet light-induced keratinocyte carcinoma of the skin, suggesting modulation of the skin metabolome by tomato bioactives. Outside of carotenoids however, other tomato phytochemicals have not been well studied in this context. The objective of this study was to use untargeted metabolomics to profile and compare the skin metabolomes of mice fed control and tomato-containing diets to elucidate additional putative bioactive compounds responsible for the observed reduction in tumor number. Male hairless and immunocompetent SKH-1 mice were fed an AIN-93G diet, an AIN-93G diet + 10% freeze dried red tomato powder (high in all-trans-lycopene) or 10% tangerine tomato (a unique tomato variety high in cis-lycopene and lycopene precursors) powder for 35 weeks. Methanolic extracts of skin were analyzed to compare differences in metabolite profiles between the control and tomato fed mice, focusing on compounds derived from tomatoes and their in vivo metabolites. By employing UHPLC-QTOF-MS-based untargeted metabolomics, we were able to detect over 6,000 compounds in skin, and distinguish metabolite profiles based on diet type using multivariate statistics. An understanding of the metabolomic changes in skin with tomato supplementation can generate testable hypotheses to better understand ways in which this fruit may be exerting protective effects.

**Poster #: 481**

**Abstract #: 2337**

**Abstract Title:** MS-based analysis of rye bran phytochemicals and their microbial metabolites

**Authors:** Ville Koistinen, Emilia Nordlund, Marko Lehtonen, Kati Katina, Kaisa Poutanen, Anna-Marja Aura, Kati Hanhineva,

**Presenting Author Affiliation:** PhD student

**Abstract Submission:**

Phytochemicals, plant-based bioactive compounds, are abundant in whole grains. According to the prevailing hypothesis, a complex mixture of phytochemicals works in synergy to partly account for the reduced risk of major non-communicable diseases, which has been associated with a high intake of plant-based foods, including whole grains. Rye (*Secale cereale*) is one of the major cereals cultivated in Europe compared with wheat, it may have additional health implications, thus promoting its use in the diet. Rye bran is rich with dietary fibre, also containing phenolic acids and other phytochemicals within its matrix.

We investigated the phytochemical profile of four different wheat and rye breads and the effect of rye bran bioprocessing on the profile. In another study, we examined the metabolism of phenolic acids in an *in vitro* model of the human gastrointestinal tract. Two types of wheat bread, fortified with native or bioprocessed rye bran, were digested in the model. In both studies, the samples were analysed with mass spectrometry coupled with liquid chromatography (UPLC–ESI–qTOF), and in the *in vitro* study, additionally with two-dimensional gas chromatography (GC×GC–TOFMS).

The bioprocessing of rye bran induced changes in the phytochemical profile of breads dissimilar to sourdough fermentation. A high release of phenolic acids from the bran matrix, deglycosylation of benzoxazinoids and a moderate degradation of alkylresorcinols was observed. In the *in vitro* experiment, rye bran induced a rapid formation of phenolic end products, native bran having more resilience than bioprocessed bran in the release and conversion of some of the compounds. Rye bran thus affected the diversity and time course of the *in vitro* metabolism of phenolic acids by human colonic microbiota, indicating that rye bran and its processing have an impact on the composition of these compounds entering the circulation.



**Poster #: 482**

**Abstract #: 2357**

**Abstract Title:** SOAP-NMR, a pre-treatment strategy for <sup>1</sup>H-NMR data in R

**Authors:** Manon Martin, Benoît Legat, Pascal de Tullio, Bernadette Govaerts,

**Presenting Author Affiliation:** ISBA - Université catholique de Louvain

**Abstract Submission:**

Metabolomic studies always result in the analysis of complex and megavariable datasets. Among the high-throughput analytical technologies, the <sup>1</sup>H-NMR spectroscopy generates 1D raw FIDs with more than 30.000 points containing the informative signal but also uncontrolled variability and artefacts. Heavy pre-treatment is required to ensure informative and interpretable spectral profiles linked to the biological perturbation under study. However, many challenges remain related to its methodology and implementation: common pre-treatment procedures require manual adjustments, they mostly remain elementary and arbitrary. Even worse, they lack repeatability and quality criteria. Besides, no clear guidelines nor global strategy based on an integrated pre-treatment system are available.

We present SOAP-NMR, a semi-automated pre-treatment strategy available in R with advanced pre-processing steps for <sup>1</sup>H-NMR data from biofluids. It includes recognised methods and in-house algorithms to outperform the current pre-treatment scheme. The library works as an open system with full flexibility: each block of pre-treatment can be performed independently yet it can provide a full advised series of steps easily implemented sequentially. These steps will operate on the signal/spectrum in order to suppress artifacts (e.g. baseline correction with asymmetric least squares), increase the Signal-to-Noise ratio (apodization), correct for biologically related problems such as peak shifts (e.g. semi-parametric time warping) or the presence of solvent residuals signal (with penalised least squares) and transform the data domain and scale (e.g. fourier transform, ppm conversion). Everyone knows that pre-treatment can also deteriorate the spectral information. Therefore, it is important to define validation tools to compare pre-treatment strategies on a same set of samples. Here, quantitative quality criteria based on spectral repeatability are derived from the Metabolomic Informative Content concept and include inertia measures, groups' homogeneity and quality indexes for clustering and the Q2 PLS-DA. Preliminary results showed that SOAP-NMR outperforms common pre-treatment procedures on the basis of spectral repeatability criteria.

**Poster #: 483**

**Abstract #: 2483**

**Abstract Title:** CAN 2 APPLES A DAY DECREASE CHOLESTEROL AND MODULATE THE GUT MICROBIOME IN MILDLY HYPERCHOLESTEROLAEMIC SUBJECTS?

**Authors:** Athanasios Koutsos, Marynka Ulaszewska, Francesca Fava, Jan Stanstrup, Kajetan Trost, Letizia Mariani, Amanda Galvin, Tanya Braune, Fulvio Mattivi, Julie A. Lovegrove, Kieran Tuohy,

**Presenting Author Affiliation:** Fondazione Edmund Mach

**Abstract Submission:**

Apples are a rich source of polyphenols and fiber. An important proportion of these bioactive components escape digestion in the upper intestinal tract and reach the colon where they can be transformed by the gut microbiota. A randomized, controlled, crossover, intervention was performed (AVAG-AGER study) to test the hypothesis that 2 apples a day can beneficially modulate the gut microbiome and cardiovascular health in mild hypercholesterolaemic subjects.

Forty volunteers consumed 2 apples (Renetta Canada variety) or 100 ml of a sugar matched control drink, daily for 8 weeks separated by 4-week washout period. Blood, urine and faecal samples were collected before and after each treatment.

We combined targeted and untargeted analytical strategies for metabolomic fingerprinting of body fluids. LC-HRMS Orbitrap was used for untargeted assays to identify the putative biomarkers of intake and for the validation of apple consumption markers. Additionally, targeted assay with use of UHPLC-MS/MS was employed for bile acids and carnitines quantitative profiling with isotopic dilution method. Changes in faecal populations were identified using fluorescence in situ hybridization (FISH) and 16S rRNA gene profiling

Preliminary results show a significant time x treatment interaction for total cholesterol, LDL-cholesterol and vascular cell adhesion molecule-1 with lower concentrations after apple intake compared to both, the baseline measurements and the control apple juice. Metabolomics analysis allowed to further investigate the compliance and the putative mechanisms of actions, with the identification of a number of dose dependent biomarkers – including various microbial classes of apple polyphenols., as well as significant changes of bile acids in plasma of treated volunteers. FISH and 16S rRNA analysis indicates a small change in selected bacterial groups for the same group of subjects.

Consuming 2 apples a day may in conclusion beneficially affect cardiovascular health and modulate both microbial composition and metabolic output.

**Poster #: 484**

**Abstract #: 2562**

**Abstract Title:** GC-MS analysis of organic acids in human urine using ethyl chloroformate as a derivatization agent

**Authors:** Pedapati S.C. Sri Harsha, Lorraine Brennan,

**Presenting Author Affiliation:** University College Dublin

**Abstract Submission:**

There is an increasing interest in measuring organic acids in biological samples such as urine. The aim of this work was to develop a fast and reliable method for profiling organic acids in human urine. The method developed is based on rapid esterification of carboxylic acids in aqueous pyridine-containing media with ethyl chloroformate used as derivatization agent. The reaction conditions were standardized for various analytical parameters and optimal results were obtained at a pH range of 8-9 and inlet temperature of 260 C. Initial GC oven temperature was held at 80 C for 4 min, ramped to 110 C at a rate of 4 C min<sup>-1</sup>, to 140 C at a rate of 10 C min<sup>-1</sup>, to 240 C at a rate of 10 C min<sup>-1</sup>, to 280 C at a rate of 4 C min<sup>-1</sup>, and then held at 280 C for 3 min. For development of the method, a total of ten standard organic acid compounds were used. All 10 standards had an acceptable linearity at concentration range of 1-50 mg/L. The injections were performed in triplicate for each concentration. The correlation coefficient ( $r^2$ ) was >0.994 for all the analysed standards. The instrument repeatability was evaluated at 25 mg/L for all the standards. The average RSD% for these measurements was 1.83 with a range of 0.42–3.16 %. Application of the method to human urine led to the identification of 35 metabolites. While the current protocol serves the purpose for the screening of organic acids in human urine, our future line of study will focus on other analytical method validation parameters such as method repeatability, stability, recovery and quantification of urinary metabolites to achieve better method evaluation.

**Poster #: 485**

**Abstract #: 2567**

**Abstract Title:** Effect of healthy Nordic diet on plasma and urine metabolic profiles – a multicentre randomized dietary intervention, SYSDIET

**Authors:** Gözde Gürdeniz, Ursula Schwab, Fredrik Rosqvist, Lieselotte Cloetens, Marjukka Kolehmainen, Lea Brader, Kjeld Hermansen, Kaisa S. Poutanen, Janne Hukkanen, Markku J. Savolainen, Matti Uusitupa, Björn Åkesson, Inga Thorsdottir, UI

**Presenting Author Affiliation:** Department of Nutrition, Exercise and Sports, Univ

**Abstract Submission:**

**Background:** A healthy Nordic diet (HND) is associated with improved lipid profile and shown to have beneficial effects on low-grade inflammation in subjects with metabolic syndrome.

**Objective:** The aim of this study is to elucidate the effect of an isocaloric HND on plasma and urine metabolic profiles in subjects with some features of the metabolic syndrome.

**Methods:** In a randomized dietary intervention, 200 overweight subjects (mean age 55, females 67%) were recruited in 6 centres across the Nordic countries. Subjects were randomised either to HND including whole-grain products, berries, fruits and vegetables, rapeseed oil and fish) or the control diet (average Nordic diet, AND). Among 166 completers, fasting plasma and 24h urine samples from 92 HND and 67 AND subjects were collected and metabolites profiled by UPLC-QTOF. PLSDA was used to discriminate the two diets and VIPs were evaluated to identify the group of metabolites reflecting the differences between two diets groups. PLSDA models were validated with test set misclassifications and AUROC.

**Results:** PLSDA led to selection of 71 and 16 metabolites for urine and plasma, respectively. Using only selected metabolites, test set misclassification were 18 % for urine and 22 % plasma. Some of the urine metabolites associated with the HND were markers of berry intake. A furan fatty acid metabolite, CMPF, was elevated in both urine and plasma after consumption of the HND. Furthermore, several classes of plasma phospholipids containing MUFA's and PUFA's increased after the HND.

**Conclusions:** Consumption of HND reflected the metabolites associated with the intake of berries and fish (CMPF) in urine. In plasma the HND specifically increased the concentrations of MUFA and PUFA containing phospholipids. These features indicate that compliance with the two diets was good and that markers reflecting the defined dietary changes were the main discriminant features in both plasma and urine.

**Poster #: 486**

**Abstract #: 2581**

**Abstract Title:** A multi-omics approach to characterize the metabolic effects of fermented dairy products on healthy men

**Authors:** Gregory Pimentel,

**Presenting Author Affiliation:** Agroscope

**Abstract Submission:**

Humans have fermented foods for over 7'500 years, first to increase shelf life, then to improve taste. This tradition now translates into dietary patterns in which up to a third of human diets is made of fermented foods, not the least because of their potential impact on health. Dairy products represent a major fraction of fermented foods in western societies. Characterizing their interaction with humans requests a holistic approach that takes into account the food, lactic acid bacteria (LAB), and the human host.

A double-blinded, cross-over clinical intervention was conducted on fourteen healthy men fed a yoghurt containing the widely used probiotic *Lactobacillus rhamnosus* GG and a non-fermented milk. Mass spectrometry-based untargeted metabolomics was conducted on blood serum of the subjects to assess their postprandial response as well as their fasting status after a two-week intervention. The postprandial metabolome of the subjects after a high-fat metabolic challenge, known to induce a transient inflammatory response, was also evaluated at the end of the intervention phase. Clinical chemistry and quantification of the faecal microbiota completed the analyses of the human samples. Finally, the metabolome of the dairy products as well as the metagenome of the yoghurt were measured.

The serum metabolomes were able to differentiate milk from yoghurt ingestion. In particular, we have identified regulated endogenous metabolites as well as products of milk fermentation that are transferred to human blood. Some of the metabolites differentiating the consumption of the two products under postprandial conditions were also discriminative under fasting conditions after two-week intervention. Finally, a significant reduction of the inflammatory response associated with the high fat meal challenge was observed.

Taken together, this multi-omics work would allow us to link the genome of the fermenting LAB, the metabolome of the test products, as well as metabolic and immunomodulatory parameters of the subjects.

**Poster #: 487**

**Abstract #: 2590**

**Abstract Title:** Community-based urine sampling integrating biomarker technology for patient health assessment

**Authors:** Manfred Beckmann, Amanda Lloyd, Naomi Willis, Long Xie, Hassan Zubair, Kathleen Taillart, John C Mathers, John Draper,

**Presenting Author Affiliation:** Aberystwyth University

**Abstract Submission:**

Dietary exposure and nutritional status has a huge impact on the health and well-being of populations. It is widely accepted that there is great potential to use dietary advice/interventions to delay drivers of chronic health risk in individuals. Obtaining objective, self-reported dietary exposure information from individuals is challenging as a result of the complexity of the monitoring tools (e.g. Food Frequency Questionnaires, diet diaries), bias and genuine miss-reporting, hindering research efforts to link specific foods to a clear health phenotype. In epidemiological studies, spot urines are a non-invasive, easy sample for participants to collect in their home-settings. It is essential that the methodology has minimal impact on the day-to-day activities of participants, and is data rich and robust in terms of population monitoring.

We have developed and validated a simplified, robust, diagnostic urine population screening method using a home urine sampling kit. Two multiple reaction monitoring (MRM) routines using triple quadrupole mass spectrometry (QQQ-MS) have been developed to quantify concurrently dietary exposure biomarkers of more than 20 foods of high public health importance in the UK. A high level of compliance was achieved by participants in collection of spot urine samples using a bespoke home urine sampling kit without supervision. The metabolite signals representing selected dietary exposure biomarkers showed no signs of degradation after prolonged storage at room temperature, +4°C, -20 °C and -80 °C to ensure the stability of samples collected in by participants in a near epidemiological setting. It is expected that method will be acceptable in a wide variety of community-based situations and is suitable for routine use to cheaply and objectively monitor behavioural (e.g. diet and exercise) drivers of chronic health risk and can provide dietary exposure information on current health status much closer to point of care.

**Poster #: 488**

**Abstract #: 2172**

**Abstract Title:** Exploring the impact of consuming different types of meat on metabolome profiles using a GC-MS metabolomics approach

**Authors:** Hiroshi Tatano, Hisami Yamanaka-Okumura, Daisuke Kajiura, Chika Kondo, Masashi Masuda, Akiyoshi Hirayama, Kazuaki Mawatari, Yoshichika Kawai, Yutaka Taketani,

**Presenting Author Affiliation:** Department of Clinical Nutrition and Food Manageme

**Abstract Submission:**

Dietary intake is traditionally assessed based on self-report dietary questionnaires, with under reporting being one of the main problems. Meat consumption affects an individual's health and nutritional status therefore, a biomarker is required to objectively and accurately evaluate the consumption of meat. We investigated whether the profiles of postprandial plasma and urine metabolites are influenced by the consumption of different types of meat in healthy subjects.

In a randomized crossover study, 8 healthy male subjects alternately consumed one of three test foods, including 1) 100 g of beef, 2) 100 g of pork, or 3) 100 g of chicken, each with a cup of water (150 ml) on a different day. Plasma and urine samples were collected before and 2 h after the consumption. Samples were analyzed with a focus on amino and organic acids using GC-MS, followed by multivariate data analysis.

This analysis revealed a marked separation between the metabolite profiles before and after the consumption in both plasma and urine. To provide pairwise comparison between meats, OPLS-DA models were generated. As a result, in the plasma samples, we identified 5 metabolites (including L-glutamine) to be the biomarker candidates for the consumption of beef, 4 metabolites (including L-lysine) for the consumption of chicken, and 13 metabolites (including L-serine) for the consumption of pork. In the urine samples, we identified 6 metabolites (including  $\beta$ -hydroxyisovalerate) to be the biomarker candidates for the consumption of beef, 7 metabolites (including 3-methyl-L-histidine) for the consumption of chicken, and 13 metabolites (including p-cresol) for the consumption of pork. However, no significant differences among the concentration of metabolites after the ingestion of beef, pork, and chicken were observed based on the biomarker candidates.

In a healthy person, the consumption of different types of meat resulted in the respective profiles of postprandial plasma and urine metabolites.

**Poster #: 489**

**Abstract #: 2570**

**Abstract Title:** Interspecies Response to Drought: in a Seed Perspective

**Authors:** Lingling Wen,

**Presenting Author Affiliation:** Ben-Gurion University

**Abstract Submission:**

The seed is the main organ responsible for the evolutionary upkeep of the plant lineage. Moreover, seeds represent the marketable yield component in important crops as food and feed. In a changing climate, drought events can have significant effects on crop yield reducing the plant productivity in regions characterized by a Mediterranean like climate. Thus a thorough understanding of the implications of water deficit on seed development and metabolism is necessary for quality seed production.

Here, we evaluated communal and species-specific metabolic alterations caused by drought stress via univariate and multivariate approaches based on gas chromatography coupled with mass spectrometry (GC-MS) data in seeds of different cultivars in the following crops: maize, sunflower, eggplant, pepper, tomato, melon and watermelon. Morphological data for traits of seeds and fruits and yield was also collected. The plants were grown in randomized block in Akko Experiment Station, Israel during 2012. Drought and well-water (regarded as control) treatments were applied to the plants in three replicates.

Univariate analyses of the metabolite profiles of mature seeds revealed that (1) extensive species diversity in the seed metabolism linked to the plant family, (2) significant species dependent differences between varieties, (3) species specific stress response metabolism. Also the effects of drought on morphological traits and yield were inconsistent among the different crops except for plant weight. Correlation-based network revealed that, seeds have species-specific metabolic responses to stress, suggesting a metabolic based phylogenetic link between species. The results of combined correlation between metabolite profiling and morphological traits can be a powerful approach to understand the mechanism of drought response evolution in plants and provide the basis for the development of new strategies to enhance crop tolerance to drought.



**Poster #: 490**

**Abstract #: 2263**

**Abstract Title:** Untargeted Metabolomics reveals novel biomarkers for Parkinson's disease subtype differentiation

**Authors:** Daniel Stoessel,

**Presenting Author Affiliation:** PhD Student

**Abstract Submission:**

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder after Alzheimer's disease with about 4.6 million cases worldwide (2005) and is caused by the degeneration of dopaminergic neurons, e.g. in the substantia nigra. The diagnosis of PD is still challenging since it rests entirely on clinical features. These features show limitations especially for the differentiation between different PD subtypes, such as tremor-dominant and the akinetic rigid subtypes. Since the determinants of the described subtypes are unknown, we pursued an untargeted metabolomics approach to measure serum metabolite profile patterns of 40 healthy controls, 40 tremor dominant PD and 40 akinetic rigid PD patients.

Metabolomic profiles show a clear and specific separation between the described subtypes and revealed promising metabolic biomarkers for subtype differentiation. While tremor-dominant PD and controls are not clearly distinguishable, akinetic-rigid PD and controls are. Surprisingly, the differentiation between akinetic-rigid and tremor-dominant PD was relatively pronounced. Most informative metabolite markers comprise different glycerophospholipids and sphingolipids including species that have already been described to be associated with PD and metabolites indicating differences in oxidative stress response. The application of classifications models such as random forest and partial least square discriminant analysis (PLS-DA) enabled us to build predictive models to classify specific subtypes with an error rate below 15%. Several potential biomarkers could be identified with an area under the curve (AUC) of >0.9. Overall, our study revealed promising novel biomarkers which may be used to differentiate the described PD subtypes. Results are currently validated in an independent sample.