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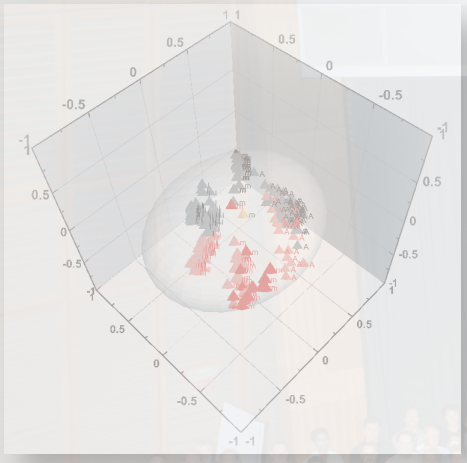

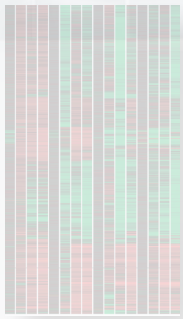

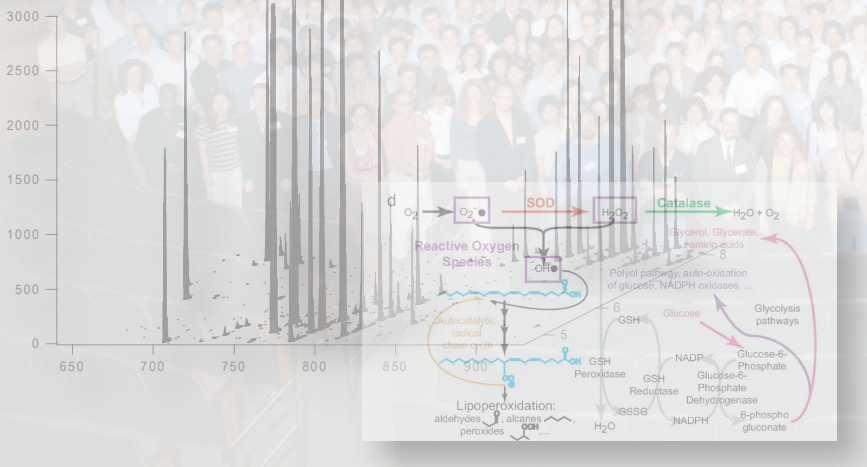

Metabolomics Society's 4th Annual International Conference

2-6 September, 2008

Boston Park Plaza Hotel
Boston, Massachusetts, USA

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METABOLOMICS SOCIETY

BOSTON 2008

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WELCOME TO METABOLOMICS 2008

It is our great pleasure to welcome you again to the historic city of Boston and to our 4th annual meeting of the Metabolomics Society. It is wonderful to see you come from countries far and near, from the public and the private sectors, from academic institutions, industry, government, and regulatory agencies. We promise you stimulating days of workshops and meetings where members of our community will share the newest innovations in our field and their impact on medical diagnoses and treatment of complex diseases, many which have no effective therapies.

Four years ago a few people sat around a table one evening and thought it was time to launch a society and a journal focused on a new concept, termed by some metabolomics, but equally referred to by others as metabonomics and metabolic profiling. We were confident that the time was right that this global science of biochemistry was going to have far reaching applications. You all ventured with us and together we created three highly successful meetings in Japan, the US, and England. We became home for the Metabolomics Journal, a journal we are proud of. We hosted workshops to educate on concept of metabolomics and we increased our membership to close to 500 members. We now look forward to our elections due in September at which time current officers will step down and new board members will be elected. Then, members of the new and old boards will collectively elect new officers for the society. Please make sure that you attend our dinner banquet on Friday during which we will review the elections process. We urge you to participate in the elections so that you ensure that the right leadership will be on board to lead the next growth phase of the society.

On a personal note, it was extremely gratifying to work with all of you to build the current solid foundations of our society. It is thanks to you all that many successes were created to put the society on the map. I am very grateful to all board members who worked very hard on behalf of the community. Special thanks go to Dr. Oliver Fiehn who worked hard on our scientific program and to Dr. Lloyd Sumner who helped us raise the funds needed for this meeting. Many thanks go to all the sponsors who made our event a reality. Last but not least, thank you Anita Howard and Eddy El Khoury for putting the meeting's organizational details together.

We hope that this meeting will offer you a chance to make new colleagues in the field of metabolomics, and meet and refresh old friendships. We also hope that during this meeting you will explore the beautiful city of Boston, cradle to much of US history, science and culture. Please feel free to consult with the front desk regarding details for exploring Boston highlights, tours, events, points of interest. But most of all don't miss on the unique scientific program that we have in store for you and on the opportunity to actively participate in advancing the science that is the driving force behind this conference.

Welcome to Boston!

Rima Kaddurah-Daouk
President, Metabolomics Society
Duke University Medical Center

WELCOME LETTER FROM THE PROGRAM COMMITTEE CHAIR

Dear Participants,

As the Program Chair for the 4th Annual Metabolomics Society Conference in Boston, I'd like to welcome you to the meeting and hope that you will enjoy great interactions with metabolomic scientists from a broad range of disciplines. The meeting brings together a variety of teams from government, commercial, and academic organizations worldwide to foster cooperation and coordination in comprehensive analysis of metabolism, to increase our understanding of metabolic regulation in a broad range of organisms, and to improve our technologies and databases to investigate biological responses. As always, we also extend a warm welcome to all our colleagues in industry who share our interest in improving the science of metabolomics.

In addition to the great program, the conference provides an excellent environment to meet your peers in metabolism studies, build relationships, and exchange lessons learned. You will certainly notice that our 2008 emphasis was placed on understanding the role of metabolism in a variety of (human) disease areas, although we have also extended to technology and systems biology sessions which cover aspects of microbial metabolism. We have deliberately not focused on plant metabolism as our partners in Plant Metabolomics have hosted an excellent conference in Yokohama, Japan in July 2008, which was supported by the Society. It is with great pleasure to announce that the attendees of the biannual Plant Metabolomics meeting have voted with a great majority to bring together the two conferences in a single joint convention that is planned for 2010 in the Netherlands.

The 2008 conference program is an exciting combination of biological applications and technology sessions. Our key note speakers include Dr Gregory Stephanopoulos from the MIT and Dr Shankar Subramaniam from UC San Diego, who will both speak about the huge role of bioinformatics in current metabolomic research. Through these lectures and throughout the program, it is becoming increasingly clear that metabolomics is not just 'measuring more metabolites' but that understanding metabolism in a comprehensive way requires seamless integration of a variety of sciences and disciplines, and hence, collaboration between many scientists. This is what our conference wants to achieve! I specifically want to thank the organizing committee, the session chairs and the three boards of the Society for selecting oral presentations and for putting together this exciting program.

As one of the most urban cities of the U.S., first time visitors to Boston are usually overwhelmed by the sheer beauty of the city. Boston is a city that manages to surprise and invites to have a splendid walk through the historic districts. I look forward to meeting you in Boston for a truly rewarding week!

Dr. Oliver Fiehn
Program Committee Chair

Workshop and Conference Programmes

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WORKSHOP PROGRAMME

Day 1: Tuesday 2nd September 2008

Session 1: Mass Spectrometry 'Fundamentals and New Development'

Chair: Lloyd W. Sumner, Noble Foundation

16.30 – 17.00 Gary Siuzdak, Scripps
'LC/MS Metabolomics :Toward Comprehensive Metabolite Analysis'

17.00 – 17.30 Oliver Fiehn, UC Davis Genome Center
'Quality control in GC/MS based metabolite profiling'

17.30 – 17.50 John Shockcor, Waters
'Analysis of Lipids by TAP Fragmentation using a Hybrid Quadrupole/Travelling Wave Ion Mobility/oa-TOF Mass Spectrometer'

17.50 – 18.10 Theodore Sana, Agilent
'A strategy for increased metabolome coverage in erythrocytes using a single LC chromatographic method with multi-modal LC/MS detection'

18.10 – 18.30 Ron Bonner, Applied Biosystems
'Unexpected variance: Artifacts and how to find them'

Day 2: Wednesday 3rd September 2008

Session 1: Mass Spectrometry 'Standardized librarie MS/MS and MS'

08.30 – 09.00 Thomas Hankemeier, Netherlands Metabolomics Centre
'Identification of metabolites using MS/MS strategies'

09.00 – 09.30 Robert Mistrik, HighChem Slovakia
'A cool outlook on uncool fragmentation processes'

09.30 – 10.00 **Chair**: Oliver Fiehn, UC Davis Genome Center
'GC/MS libraries: comparing quadrupole and TOF mass spectrometry'

Session 2: Data Analysis

10.45 – 11.05 **Chair**: Roy Goodacre, University of Manchester, Manchester UK
'Multivariate statistical analyses and machine learning'

11.05 – 11.30 Richard Brereton, U Bristol, UK
'Sampling designs and data preprocessing'

11.30 – 11.55 Andreas Vidman, Umetrics, Sweden
'Projections methods for analysing metabolomic and other -omic data'

11.55 – 12.15 Chair: Bruce Kristal, Harvard Medical School
'The risk of data overfitting'

12.15 – 12.35 Christine Des Rosiers, U Montreal, Canada
'From isotopomer data to metabolic flux ratio calculations: Basic guidelines'

WORKSHOP PROGRAMME

Session 3: NMR 'Principles and Pitfalls'

14.30 – 15.00	**Chair**: Jules Griffin, U Cambridge, UK *'Pulse sequences and spectral editing for NMR based metabolomics'*
15.00 – 15.30	Gerhard Wagner, Harvard Medical School *'NMR approaches for indentification of metabolite changes due to expression of oncogenes'*
15.30 – 16.00	Siva Sivakolundu, Bruker BioSpin *'High Throughput Metabolic Profiling of Tissues using hr-MAS NMR'*

Session 4: Sample Preparation

14.30 – 15.00	**Chair**: John Newman, USDA Western Human Nutrition Center *'Quality Control Principles in Sample Preparation'*
15.00 – 15.30	Wayne Matson, Bedford VA Medical College *'Affect of Sample Preparation Pitfalls in Human Clinical Studies'*
15.30 – 16.00	Dan Jones, Michigan State *'Simplification and Cost Reduction in Large-scale Sample Processing'*

CONFERENCE PROGRAMME

Day 1: Wednesday 3rd September 2008

Session 1: Keynote Lectures 'Metabolism: The Grand View'

16.30 – 17.00 **Chair**: Rima Kaddurah-Daouk, Duke University

17.00 – 17.45 Gregory Stephanopolous, MIT
'Transcriptional, Metabolomic and Flux data: What are they good for?'

17.45 – 18.30 Shankar Subramaniam, UC San Diego

'Bioinformatics approaches to regulation of lipid metabolism'

Day 2: Thursday 4th September 2008

Session 1: Cancer Preclinical and Clinical Research

08.30 – 09.00 **Chair**: Sudhir Srivastava, National Cancer Institute
'Metabolomics: A Bridging Omics between the Normal and Cancer Phenotypes'

09.00 – 09.30 Zaver M. Bhujwalla, John Hopkins, School of Medicine
'Molecular and Functional Imaging of Cancer'

09.30 – 09.50 Andrew Lane, U Louisville
'The different uses of glucose and glutamine in cancer cells'

09.50 – 10.10 Facundo Fernandez, Georgia Tech U
'Ovarian cancer serum metabolomics by LC/TOF MS and direct analysis in real time (DART) / TOF MS'

10.10 – 10.30 Jeff Schuster, Metabolon
'Investigation of prostate cancer aggressivity using metabolomics'

Session 2: Spatially Resolved Metabolite Imaging and Flux Analysis

Chair: Lloyd W. Sumner, Noble Foundation

11.00 – 11.30 Richard M. Caprioli, Vanderbilt Cancer Center
'Molecular Profiling and Imaging of Tissues by Mass Spectrometry: Assessing Spatial and Temporal Proteomic'

11.30 – 12.00 Gary Siuzdak, Scripps
'Nanostructure-Initiator Mass Spectrometry (NIMS) Metabolite Imaging'

12.00 – 12.20 Teresa Fan,
'Altered Regulation of Metabolic Pathways in Human Lung Cancer Discerned by ^{13}C-Isotopomer Profiling'

12.20 – 12.40 WN Paul Lee, UC Los Angeles
'Amino acid synthesis in HepG2 cells: insight from isotopomer analysis'

12.40 – 13.00 Johanna Scarino, Princeton
'Active metabolism in fibroblasts after exiting the cell cycle'

Session 3: Nutrition and Epidemiology

Chair: Bruce Kristal, Harvard Medical School

14.30 – 15.00 Walter Willet, Harvard Medical School
'The role of non genetic factors in chronic disease: no shortage of space for metabolomics'

15.00 – 15.30 Bruce Kristal, Harvard Medical School
'Validated biomarkers of caloric restriction in rats: Markers of disease risk in humans?'

15.30 – 15.50 Gregor McCombie, U Cambridge, UK
'A combined NMR and mass spectrometry metabolomic study of weight loss and omega-3 oil intake determines selective short chain triglyceride reduction'

15.50 – 16.10 Elin Chorell, Umea, Sweden
'Nutrition-modulation by predictive metabolomics'

16.10 – 16.30 Christopher Crutchfield, Princeton
'The metabolic response of yeast to nutrient limitation'

Session 4: Cardiovascular Disease

Chair: Robert Gerszten, Massachussetts Gen. Hosp.

17.30 – 18.00 Ron Krauss, Children's Hospital Oakland
'Metabolomic Analysis of variation in Response to Statin Treatment'

18.00 – 18.30 Robert Gerszten, Massachussetts Gen. Hosp.
'Towards a Metabolomics Platform for Cardiovascular Disease: Insights from planned Heart Attacks'

18.30 – 18.50 Kian-Kai Cheng, U Cambridge, UK
'A metabolomic study of a mouse model of atherosclerosis'

18.50 – 19.10 Christine Des Rosiers, U Montreal, Canada
'Isotopomer analysis as applied to the heart: From metabolic flux analysis to therapeutic strategy'

19.10 – 19.30 Hans-Peter Deigner. Biocrates, Austria
'Targeted Metabolomics on Age-dependent and Stroke-induced Lipids'

Day 3: Friday 5th September 2008

Session 1: Diseases of the Central Nervous System

Chair: Rima Kaddurah-Daouk, Duke U

08.30 – 09.00 Steven Hersch, MIND Institute, Massachusetts Gen. Hosp.
'Huntington's disease: a central neurodegenerative disorder reflected in the peripheral metabolome'

CONFERENCE PROGRAMME

09.00 – 09.30 Rima Kaddurah-Daouk, Duke U
'Metabolomics of Neuropsychiatric Disorders'

09.30 – 09.50 William Wikoff, Scripps
'Using metabolomics of animal models to understand complex human biochemistry and disease'

09.50 – 10.10 Marlon Quinones, UNC Chapel Hill
'Exploring the impact of lithium treatment on the metabolome: A global biochemical dissection of treatment-response correlates in bipolar disorder'

10:10 – 10:30 Reza M Salek, U Cambridge, UK
'A metabolomic comparison of mouse models of Neuronal Ceroid Lipofuscinoses'

Session 2: Databases and Metabolite Identification

Chair: Oliver Fiehn, UC Davis Genome Centre

11.00 – 11.25 Steve Bryant, Natl. Center for Biotechnology Information
'PubChem, A Public Repository for Chemical Biology Screening Results

11.25 – 11.50 Robert Mistrik, HighChem, Slovakia
''Integrated Approach for Structural Analysis of Endogenous Metabolites'

11.50 – 12.10 Tobias Kind, UC Davis Genome Center
'Annotation of unknown metabolites with accurate mass LC-MS/MS and GC-MS by constraining metabolite database queries using analytical metadata'

12.10 – 12.30 Philip Britz-McKibbin, McMaster, Canada
'Virtual Metabolomics: metabolite identification and quantification without chemical standards'

Session 3: Diabetes

Chair: Matej Oresic, VTT, Finland
'Metabolic stress, autoimmunity, and diabetes'

14.00 – 14.30 Antonio Vidal-Puig, U Cambridge, UK
'Lipotoxicity, adipose tissue expandability and insulin resistance'

14.30 – 15.00 Matej Oresic, VTT, Finland
'Metabolic stress, autoimmunity, and diabetes'

15.00 – 15.20 Lee Roberts, U Cambridge, UK
'Metabolic phenotyping of adipocyte differentiation and peroxisome proliferator activated receptor delta activation in the 3T3-L1 cell line'

15.20 – 15.40 Ville-Petteri Mäkinen, Helsinki U Tech, Finland
'Metabolic characterization of vascular complications and their progression by proton NMR spectroscopy of serum in patients with type 1 diabetes'

15.40 – 16.00 Daniel Vis, U Amsterdam, NL
'Dynamic endocrine networks, revealing dynamic differences between health and disease'

Session 4: Networks and Pathways

Chair: Henri Brunengraber, Case Western U
'4-Phosphoacyl-CoAs, a new class of acyl-CoAs'

17.30 – 18.00	Art Castle, NIH / NIDDK *'The NIH roadmap to understand biological pathways and networks by metabolomics'*
18.00 – 18.30	Henri Brunengraber, Case Western U *'4-Phosphoacyl-CoAs, a new class of acyl-CoAs'*
18.30 – 18.50	Daniel Amador-Noguez, Princeton *'Metabolome dynamics of the glycolysis/gluconeogenesis switch in Escherichia coli'*
18.50 – 19.10	Fionnuala Morrish, Fred Hutchison Cancer Center *'Metabolites and post-translational protein modification: how oncogene regulation of the metabolome enables activation of protein signaling networks'*
19.10 – 19.30	Fangping Mu, Los Alamos National Lab *'Carbon-fate maps for metabolic reactions'*

Day 4: Saturday 6th September 2008

Session 1: Hot Topics in Microbial and Biomedical Metabolomics

Chair: David Wishart, U Alberta, Canada

08.30 – 09.00	Warwick Dunn, U Manchester, UK *'Metabolic alterations are observed in the metabolomes of plasma and placental tissue related to pre-eclampsia'*
09.00 – 09.30	André Canelas, TU Delft, NL *'Getting the right numbers: how to avoid some (common) mistakes in metabolomics-based research in S. cerevisiae'*
09.30 – 09.50	Roy Goodacre, U Manchester, UK *'Investigating abiotic stresses on biological systems using metabolic profiling, lipid profiling and spatial metabolic fingerprinting'*
09.50 – 10.10	Hui-Ming Lin, U Auckland, NZ *'Metabolite profiling of a Crohn's disease mouse model for inflammatory biomarkers'*
10.10 – 10.30	Denise Sonntag, Biocrates, Austria *'High altitude effects on the metabolism of healthy mountaineers – a quantitative metabolomics approach to medicine'*

Session 2: High Resolution Chromatography-coupled Techniques

Chair: Thomas Hankemeier, Netherlands Metabolomics Centre, NL

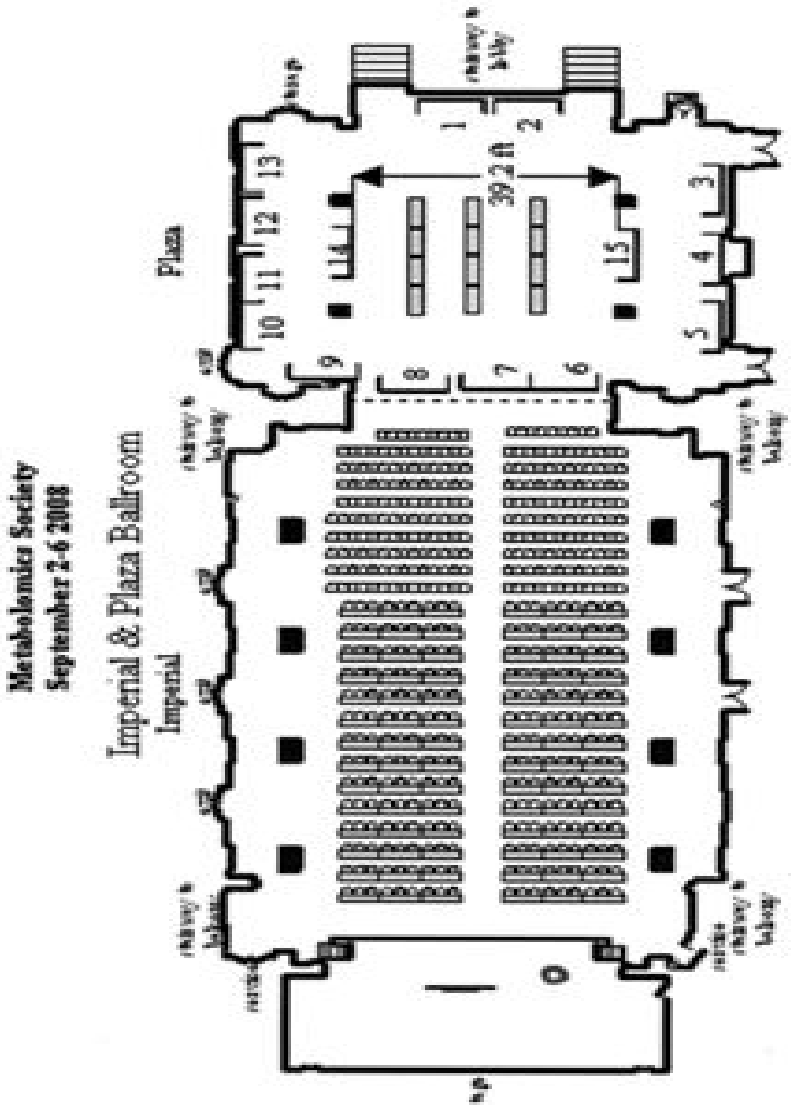
11.00 – 11.30	Gert Desmet, Vrije U, Brussels, Belgium *'Optimizing column selection, pressure and temperature for maximal peak capacity in metabolomics'*
11.30 – 11.50	Herbert Hill, Washington State U *'Metabolic Profiling of Human Blood by Ion Mobility Mass Spectrometry'*
11.50 – 12.10	Yoshiaki Ohashi, Human Metabolome Technologies, JP *'Depicting Large-Scale Metabolome Map of Histidine-Starved Escherichia coli by CE-TOFMS'*

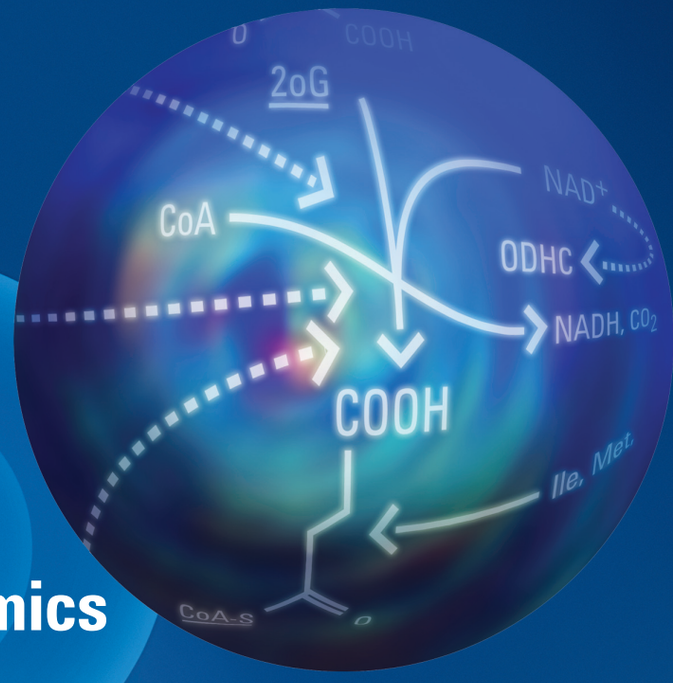
CONFERENCE PROGRAMME

12.10 – 12.30	David Watson, U Strathclyde, UK *'Applications of hydrophilic interaction fourier transform mass spectrometry in metabolomics'*
	Session 3: Drug Discovery and Drug Development
14.00 – 14.30	**Chair**: Ina Schuppe-Koistinen, Astra Zeneca *'Metabolic profiling in drug R&D: Opportunities for biomarker discovery and personalized healthcare'*
14.30 – 15.00	Bruce Hammock, UC Davis *'Case study: The soluble epoxide hydrolase inhibitors as anti-inflammatory agents'*
15.00 – 15.20	Nelly Aranibar, Bristol-Myers Squibb *'Modulation of ascorbic acid metabolism by cytochrome P450 induction revealed by a metabonomics and transcriptomics Approach'*
15.20 – 15.40	Prasad Phapale, Kyungpook National University, South Korea *'Potential of pharmacometabolomic approach to study drug response'*
15.40 – 16.00	Thomas O'Connell, U North Carolina *'The application of pharmaco-metabonomics to acetaminophen induced hepatotoxicity in humans'*
	Session 4: Systems Biology
	Chair: Minoru Kanehisa, KEGG, JP
16.30 – 17.00	Vamsi Mootha, Harvard Medical School *'Mitochondrial parts, pathways, and pathogenesis'*
17.00 – 17.30	Minoru Kanehisa, KEGG, JP *'Bioinformatic approaches to integrated analysis of genomic & chemical information'*
17.30 – 17.50	Margriet Hendriks, UMC Utrecht, NL *'Metabolic network discovery through reverse-engineering of metabolome data'*
17.50 – 18.10	Katrin Strassburg, MPI-MP, Germany *'The temperature stress response of Saccharomyces cerevisiae revisited: Integrative transcriptome and metabolome analysis based on activated metabolic networks'*
18.10 – 18.30	J Scott Breunig, Princeton *'Genetic basis of metabolome variation in yeast'*

HOTEL FLOOR PLAN

1	Springer	8	Metanomics
2	Metabolon	9	
3	Biocrates	10	Thermo Scientific
4	Bruker	11	Umetrics
5	Waters	12	Biorad
6	Chemonx	14	Agilent
7	Leco	15	ABS





Agilent in Metabolomics

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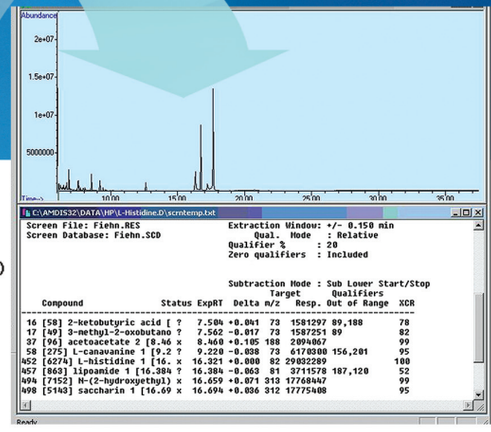

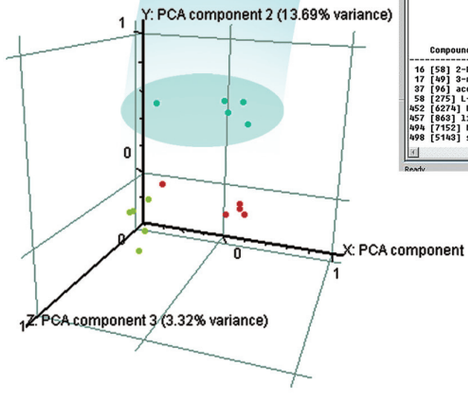

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Workshop Abstracts

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Gary Siuzdak, Scripps

'LC/MS Metabolomics: Toward Comprehensive Metabolite Analysis'

Mass spectrometry (MS) is an established technology in drug metabolite analysis and is now expanding into endogenous metabolite research. Its utility derives from its wide dynamic range, reproducible quantitative analysis, and the ability to analyze biofluids with extreme molecular complexity. The aims of developing mass spectrometry for metabolomics range from understanding basic biochemistry to biomarker discovery and the structural characterization of physiologically important metabolites. In this review we will discuss the mass spectrometry-based techniques involved in this exciting area and the current and future applications of this field.

Oliver Fiehn, UC Davis Genome Center

'Quality control in GC/MS based metabolite profiling'

It is easy and tempting to inject a lot of samples into a mass spectrometer, especially with an autosampler at hand. But how do we make sure that the quality is sufficient that all metabolites have good overall precision, that the compounds do not degrade in the injector, that the instrument is under control and that peak finding and peak intensities are consistent over time and can be repeatedly analyzed? This tutorial is meant to introduce some basic and some advanced principles of quality control, from liner exchange to syringes, internal standards, detector voltages and matrix deposition on columns. Examples are given what can go wrong, and how to check for problems.

John Shockcor, Waters

'Analysis of Lipids by TAP Fragmentation using a Hybrid Quadrupole/Travelling Wave Ion Mobility/oa-TOF Mass Spectrometer'

Ion mobility mass spectrometry (IMS) allows separation of ionic species as they drift through a gas phase under the influence of an electric field. The rate of an ions drift depends on the mass of the ion, its particular charge state and the average cross-sectional of the ion. It is possible to separate ions with the same nominal mass if they have different charge states or different interaction cross-sections.

We will describe various types of ion-mobility devices with emphasis on the use of the hybrid quadrupole/travelling wave IM/oa-TOF instrument. Examples of the application of ion-mobility mass spectrometry to metabolic studies will be presented

Theodore Sana, Agilent

'A strategy for increased metabolome coverage in erythrocytes using a single LC chromatographic method with multi-modal LC/MS detection'

Reproducible and comprehensive sample extraction, separation and detection of metabolites with a broad range of physico-chemical properties can be a highly challenging process for LC/MS. Moreover, compound matching to accurate mass libraries based on mass alone is insufficient for identification. We used human erythrocytes to demonstrate a more comprehensive sample extraction method under solvent conditions where the pH has been adjusted to pH 2, pH 6 or pH 9. Furthermore, a single binary solvent chromatographic separation method for LC/MS was developed for ESI and APCI in both ionization modes that incorporated water, methanol and acetate as a mobile phase modifier on a Zorbax SB-aq column. A total of 2,370 features (compounds and associated compound related components: isotopes, adducts and dimers) were detected across all pHs. Broader coverage of the detected metabolome was achieved by observing that (1) performing extractions at pH 2 and at pH 9, leads to a combined 92% increase in detected features over pH 7 alone; and (2) including APCI in the analysis results in a 34 % increase in detected features, across all pHs, than the total number detected by ESI only. A significant dependency of extraction solvent pH on the recovery of heme and other compounds was observed in erythrocytes and underscores the need for a comprehensive sample extraction strategy and LC/MS analysis in metabolomics profiling experiments. In addition we used the single chromatographic system to demonstrate an approach to developing an accurate mass retention time library (AMRT) that significantly improves the metabolite detection coverage and the confidence with which database matches are made. The method has the advantage of the same retention times for metabolites detected by the different ionization methods. A limited sized AMRT erythrocyte library of standards, corresponding to several common metabolites found in erythrocytes, was constructed and used to evaluate actual compound matches from the sample extracts

Ron Bonner, Applied Biosystems

'Unexpected variance; Artifacts and how to find them'

In addition to the data of interest, metabolomics data sets always contain unrelated variation that can be introduced at various points in the workflow, such as: sample handling and storage, biological variation and diversity, the analytical system, data processing, etc. This unexpected variance can confound data analysis, for example, it may incorrectly appear to differentiate sample classes or may mask real effects. This talk will show some example data and illustrate methods that can be used to identify artifacts and the variables responsible.

Thomas Hankemeier, Netherlands Metabolomics Centre
'Identification of metabolites using MS/MS strategies'

This lecture will discuss the identification of metabolites using multi-stage mass spectrometry. An introduction is given into fragmentation of metabolites using different mass spectrometers such as ion-traps, triple quad mass spectrometers, but also hybrid mass spectrometers such as combinations of ion trap or quadrupole coupled to high resolution mass spectrometers (TOF, FTMS, Orbitrap). The selection of the conditions of fragmentation experiments such as fragmentation energy, collision gas, etc, with the different instruments is important. Important aspects such as the reproducibility of multi-stage mass spectra obtained with the same MS system and between different MS systems will be considered. The advantages of using high resolution mass spectrometers and how to obtain elemental compositions will be addressed. Next, the assignment of the identity of metabolites using multi-stage mass spectra and comparing them with those present in libraries or databases will be discussed, and current databases and initiatives mentioned. In addition, the strategy how to carry out structure elucidation using multi-stage mass spectra will be addressed.

Robert Mistrik, HighChem Slovakia
'A cool outlook on uncool fragmentation processes'

Molecular ions which are sufficiently "cool" remain intact up to detection providing useful molecular weight information. However, the real power of mass spectrometry lies in its ability to produce and observe molecular decomposition products, which can assist the determination of key structural information. Even though the "uncool" fragmentation and rearrangement processes are far from random, mechanisms which govern ion decomposition pathways are not sufficiently general to be applicable to a wide range of compounds. In cases where the general rules and known mechanisms are not applicable, the fragmentation analogy of structurally related compounds may provide crucial information which reveals complex rearrangement processes. A non-thermochemical ("cool") outlook on general fragmentation rules and fragmentation analogy will be presented.

Oliver Fiehn, UC Davis Genome Center
'GC/MS libraries: comparing quadrupole and TOF mass spectrometry'

The key to metabolomics is compound identification. Without high quality criteria, claimed identifications will not be regarded trustworthy by the scientific community. Results can be interpreted in a most straightforward way for biology and medical sciences if peaks are annotated as genuine metabolites, with out links to biochemical databases and literature information. How do we achieve such reliable identifications? In GC/MS, compounds can be covered that are thermostabile and that are volatile enough (if needed, with derivatization) to sustain gas chromatography. In fact, most metabolites in the KEGG database below 500 Da are amenable by GC/MS, and that is why GC/MS presents itself as clear choice to start successful metabolomics studies.
In this tutorial, the differences between quadrupole and time-of-flight GC/MS instruments will be highlighted, and libraries are presented that are available to the scientific community. Pitfalls in compound annotation will be discussed to ensure that eventually, metabolomic reports fulfill the guidelines established by the Metabolomics Standards Initiative (MSI).

Chair: Roy Goodacre, University of Manchester, Manchester UK
'Multivariate statistical analyses and machine learning'

Richard Brereton, U Bristol, UK
'Sampling designs and data preprocessing'

Pattern recognition involves using analytical data to classify samples into groups. This presentation will show how it is possible to obtain meaningless classification results if data are not correctly obtained, preprocessed or validated. Recommendations are to compare with null simulations; to obtain adequate and balanced group sizes; to separate optimisation from validation; not to perform variable selection on both the training and test set ; to repeat test and training set splits often many hundred times to obtain stable predictions ; to suitably scale data (e.g. square root) to take into account outliers and missing or undetected variables.

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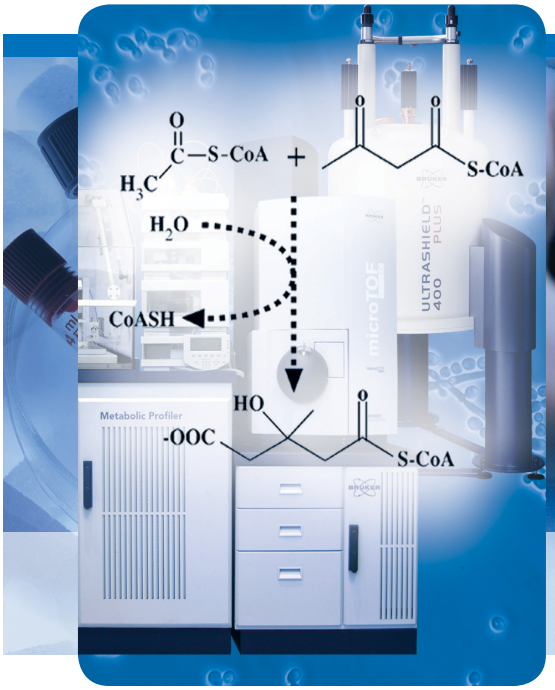

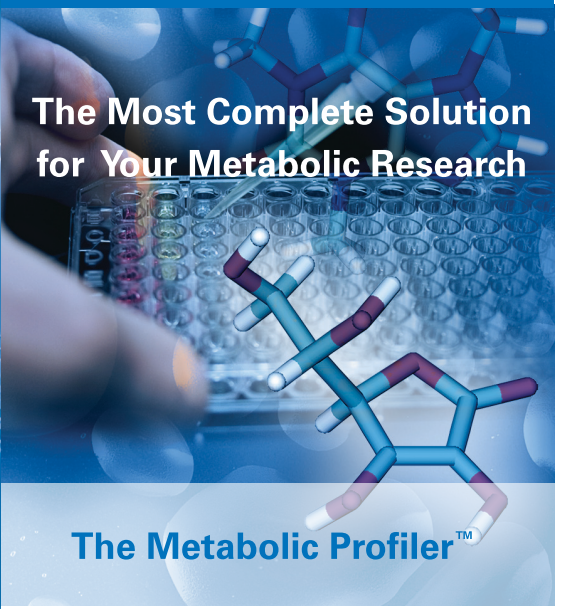

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WORKSHOP ABSTRACTS

Andreas Vidman, Umetrics, Sweden
'Projection methods for analyzing metabolomic and other "omic" data'

Omics data come from few samples (cases, patients, mice, …), typically N=10 to N=50, and very many variables (signals, peaks, …), typically K= 1 000 to K= 50 000. *Projection methods* work well with such data. These methods include principal components analysis (PCA) through PLS, OPLS and O2PLS, to discriminant analysis approaches, i.e., PLS-DA, OPLS-DA, and O2PLS-DA.

Using a few omics examples for illustration, the following will be discussed for these methods:

- Data organization
- Interpretation of the results – scores, loadings, coefficients, diagnostics
- Pertinent plots facilitating the interpretation of the underlying biological problem
- Confidence intervals, p-values and other statistics
- Missing data

Christine Des Rosiers, U Montreal, Canada
'From isotopomer data to metabolic flux ratio calculations: Basic guidelines'

Jules Griffin, U Cambridge, UK"
'Pulse sequences and spectral editing for NMR based metabolomics'

So you have 1000's of samples and you are going 'to do' metabolomics on them – well that's just hit a button and go, right? Hang on there, cowboy there is so much more your NMR spectrometer has to offer. Let's start by relaxing and using T1 and T2 filters to look at how we can detect metabolites under the humps of broader resonances. Approaches like the cpmg pulse sequence are great at 'editing out' resonances from lipids but what about large marcromolecules? Here we can probe diffusion properties, and even combine these approaches to look directly at the intracellular environment. Now that's something mass spec can't do! This is a slightly 'tongue in cheek' workshop presentation, aimed at the PhD student/new Post Doc to give an overview of options. 'Old timers' are very welcome to contribute their knowledge too!

Gerhard Wagner, Harvard Medical School
'NMR approaches for identification of metabolite changes due to expression of oncogenes'

Expression of oncogenes changes the profile of cellular metabolites. NMR spectroscopy can measure metabolite levels quantitatively. To resolve signals of many metabolites individually we use two-dimensional NMR experiments. To achieve high resolution we acquire spectra with non-linear sampling and process date with methods other than the discrete Fourier transformation. Applications to characterizing changes of metabolite levels due to over-expression of oncoproteins will be shown.

Siva Sivakolundu, Bruker BioSpin
'High Throughput Metabolic Profiling of Tissues using hr-MAS NMR'

HRMAS NMR has been found to be an excellent technique for conducting tissue profiling due to its reasonable sample requirements, limited sample processing, and quality of the data produced. Nevertheless, sample preparation remains as a limiting factor for conducting a statistically significant study using tissues. We have developed a disposable HRMAS insert to increase the sample throughput and prevent biological contamination of the rotor. This allows the efficient collection, handling, storage, and acquisition of NMR data on tissue samples from various sources. The workshop will focus of various practical aspects of conducting a tissue profiling study. New advances in the instrumentation available for HRMAS for conducting metabonomics studies on tissues will also be presented.

John Newman, USDA Western Human Nutrition Center
'Quality Control Principles in Sample Preparation'

The ability for experiments to uncover significant findings depends both on their design and data quality. By accounting for analytical biases, variance can be reduced to the biological components. Controlling variables associated with sample preparation is critical. Preparation-associated analytical variance can arise from variable target stability, extraction, derivatization, and/or resolution from interferences. These issues can often be mitigated by using internal standards, stabilizing agents/procedures, and methods to separate targets from interferences. While, no single approach is optimal for all metabolites, the issues and approaches to their mitigation are consistent, and illustrative examples will be presented.

Wayne Matson, Bedford VA Medical College

'Affect of Sample Acquisition and Processing on Metabolomic Profiles'

Protocols for sample acquisition processing and archiving have a profound effect on metabolomic profiles-particularly in multi center trials. We will discuss: A) Information content of whole blood, packed RBC, buffy coat, and plasma with respect to tyrosine, tryptophan and purine pathways, and variables of time, temperature, g force, anticoagulant and clinical acquisition protocols; B) Techniques of data analysis to compensate for site variation in archived samples; C) Strategies for multi site sample acquisition of samples. Data will be presented in the context of targeted and survey assays for biomarker discovery in CNS diseases in longitudinal, cross sectional and therapeutic studies.

Dan Jones, Michigan State

'Simplification and Cost Reduction in Large Scale Sample Processing'

Metabolite profiling studies often require analyses of large numbers of replicates owing to biological and analytical variability. Furthermore, the needs to characterize different individuals, treatments, and time points can drive costs of samples preparation and analyses to prohibitive levels. To address the needs for large-scale analysis of metabolic phenotypes, we have implemented procedures to minimize sample processing steps and mass spectrometer instrument time for targeted LC/MS/MS analyses of amino acids, structural lipids, and signaling lipids and non-target GC/MS and LC/TOF MS metabolite profiling. Some of the benefits and pitfalls of these approaches will be discussed

Invited Speaker Abstracts

Gregory Stephanopolous, MIT

'Transcriptional, Metabolimic and Flux Data: What are they good for?'

Presently, vast amounts of data are collected by modern high throughput methods begging the questions: (a) how valuable these data are, and, (b) how can one integrate them in order to identify disease biomarkers or elucidate cell physiology. Systems biology emerged as the field aspiring to provide answers to these questions. To this end, holistic methods aiming at biomarker identification for various diseases have met with considerable success. On the other hand, elucidation of cell physiology has relied heavily on classical modeling while few new approaches have been developed that are suitable for the unique requirements of systems biology. As such, success of system biology in elucidating cellular behavior has been mixed depending on the complexity of the system, quality and completeness of collected data and prior knowledge of basic mechanisms about the system.

In this talk I will illustrate these points with two examples, one for the identification of survival biomarkers of End Stage Human Disease (ESRD) and the other on the analysis of global regulation of yeast physiology. ESRD patients have high mortality rate that cannot be attributed to the usual epidemiological or clinical factors. Metabolomic analysis of plasma samples from ESRD patients has identified metabolic biomarkers that have very high sensitivity and specificity in predicting the outcome of dialysis. In the second example I will examine the integration of cell-wide measurements, such as flux and metabolomic profiling and gene expression, with networks of protein-protein interactions and transcription factor binding that has previously revealed critical insights into cellular behavior. As prior methods have essentially failed to produce acceptable correlations between metabolic flux and transcriptional data, a new approach is introduced to modeling metabolic flux dependence on transcriptional state. The new, network-based, model improved substantially flux prediction from transcription data and, in addition, facilitated the derivation of several interesting biological principles, such as, rewiring of metabolic flux by transcriptional regulation and the emergence of metabolite-enzyme interaction density as a key biosynthetic control determinant. Global transcriptional, metabolomic and flux data were used for this purpose generated in continuous cultures of yeast under stress in the absence or presence of the global regulator Gcn4p.

These results suggest that while systems biology has the potential to enhance our understanding of global cellular behavior, progress will be generally slow, ad hoc and case-dependent. There are certainly no tools that can routinely convert "omic" data to cellular knowledge, as it was initially hoped and widely expected. As such, results from comprehensive investigations are likely to not meet expectations. On the other hand, such results can only be obtained from the integrated mindframe of systems biology leading, eventually, to more realistic models of cellular regulation for understanding diseases as well as engineering strains for industrial applications.

Shankar Subramaniam, UC San Diego

'Bioinformatics approaches to regulation of lipid metabolism'

Sequencing of the human genome has opened the way and provided the impetus for building a comprehensive picture of a mammalian cell. Significant efforts are underway in the fields of genomics and proteomics to identify all genes and proteins in a given organism. The goal is a complete map of the genes, gene products and their interaction networks in a functioning cell. The next step in establishing a comprehensive picture of a cell will be to tie the cell's metabolome into the rapidly developing genomic and proteomic maps. A cell's metabolome, however, is such an enormous and complex entity that characterizing it can only be approached in sections. The LIPID MAPS Consortium (http://www.lipidmaps.org) is developing comprehensive procedures for identifying all lipids of the macrophage, following activation by endotoxin. The goal is to quantify temporal and spatial changes in lipids that occur with cellular metabolism and to develop bioinformatics approaches that establish dynamic lipid networks. To achieve these aims, an endotoxin of the highest possible analytical specification, 3-deoxy-D-*manno*-octulosonic acid $(Kdo)_2$-Lipid A, a nearly homogeneous lipopolysaccharide (LPS) sub-structure with endotoxin activity equal to lipopolysacchride (LPS, the macrophage activating factor from bacteria) was developed and used as a ligand to activate murine macrophages. Kdo_2-Lipid A is comparable to LPS by several criteria including its bioactivity, activation of TLR-4 receptor followed by well-characterized readouts such as cytokine and eicosanoid production and gene expression changes.

We report the total lipidomic response in macrophage cells to inflammatory stimulus by KDO2-lipid A from quantitative mass spectrometric measurements, transcriptional measurements and integrative data analysis. The analysis of lipidomic and concomitant transcriptomic measurements demonstrate early responses in fatty acid metabolism through increased eicosanoid cascade, later responses through increases in a large number of sphingolipids and a long term response in the biosynthesis of sterols. The analysis also demonstrates the mechanisms of lipid remodeling involving all classes of mammalian lipids, thus providing a systems-level view of lipid pathways in activated macrophages. Lipid remodeling in activated macrophages include acyl coA remodeling into phospholipids and mitochondria-driven remodeling to acetyl coA. The latter serves as a precursor for sterol biosynthesis. Pharmacological perturbations of the lipid cascade through the addition of statin drugs demonstrate both the expected sterol changes and the unexpected changes in other lipid classes. To the best of our knowledge this is most comprehensive network map of lipids in mammalian cells along with a dynamical perspective on changes associated with macrophage stimulation. The comprehensive lipidomic network, a dynamical network of lipid metabolism in activated macrophages, and the effects on this network of a pharmacological perturbation will also be presented in this lecture.

Acknowledgements: LIPID MAPS Consortium. Supported by a NIGMS Glue Grant titled LIPID MAPS.

Chair: Sudhir Srivastava, National Cancer Institute
'Metabolomics: A Bridging Omics between the Normal and Cancer Phenotypes'

Why Metabolomics Now? Recent technological advances in NMR spectroscopy, mass spectrometry, and chromatographic analysis enable direct evaluation of multiple metabolic components and analysis of metabolic fluxes. Metabolomics yields information that is both complementary and distinct from that obtained from genomics and proteomics, and metabolomics data can be integrated into the larger context of Systems Biology enabling a deeper understanding of biological processes. It can be used to monitor functional and physiological changes associated with environmental factors such as diet and drugs that influence disease process. Thus, metabolomics may be useful for diagnosis, monitoring therapeutic interventions, and detecting sub-clinical toxic effects of novel therapies and evaluating the metabolic changes involved in disease processes. While metabolomics technologies hold great promise for translational and biological research, the field is just beginning to mature into a reliable platform for translational research. The Speaker will provide an overview of metabolomics in carcinogenesis and its application in cancer detection, diagnosis, and prognosis.

Zaver M. Bhujwalla, John Hopkins, School of Medicine
'Molecular and Functional Imaging of Cancer'

Using combined MR and optical imaging of human breast and prostate cancer xenografts engineered to express green or red fluorescent protein under hypoxia, we have obtained useful insights into the dynamics between the tumor ECM, vascularization, extracellular pH, interstitial fluid transport, and metabolism. New areas being developed in our program include targeting choline kinase using siRNA delivered using lentiviral vectors, and performing image-guided targeting of choline kinase using siRNA in combination with a prodrug enzyme cytosine deaminase using a multi-modal imaging platform. These advances demonstrate the array of roles that multi-modality imaging can play in understanding and targeting tumor metabolism.

Richard M. Caprioli, Vanderbilt Cancer Center
'Molecular Profiling and Imaging of Tissues by Mass Spectrometry: Assessing Spatial and Temporal Proteomics'

The spatial and temporal aspects of molecular processes in cells and tissues play an enormous part in the biology that defines living systems. Profiling and Imaging MALDI MS provides an effective means to measure and assess those dimensions on a molecular basis, including peptides, proteins, lipids, metabolite as well as others. The technology is extraordinarily high throughput with high molecular specificity and is an excellent discovery tool. It provides the capability of mapping the location of specific molecules such as drugs, lipids, peptides and proteins directly from fresh frozen tissue sections. For example, utilization of this technology provides spatial information across a tissue section for a target protein expression or for a signature of multiple proteins and can be used to correlate changes in expression levels with specific disease states or drug response. Protein patterns can be directly correlated to known histological regions within the tissue, allowing for the direct monitoring of proteins specific to these regions within a tissue sample. Profiling and imaging MS have been used to characterize many tissue types, including human gliomas and lung cancers, as well as tumor response to specific therapeutics, suggesting the use of proteomic information in assessing disease progression as well as predicting patient response to specific treatments

Gary Siuzdak, Scripps
'Nanostructure-Initiator Mass Spectrometry (NIMS) Metabolite Imaging'

Mass spectrometry (MS) profiling of endogenous and exogenous small molecules in tissues and biofluids is a significant part of pharmaceutical development. However, tissue mass profiling by Matrix-Assisted Laser Desorption Ionization Mass Spectrometry (MALDI) has not been widely applied to small molecules because of matrix application and interference effects. Recently we have introduced Nanostructure Initiator Mass Spectrometry (NIMS) (Nature 2007), a matrix-free
platform for biomolecule analysis with minimal sample preparation. Here we show the potential of NIMS for clinical research, pharmacokinetics, and cancer tissue analysis. Overall, our results demonstrate the capacity of NIMS to perform high sensitivity and simple sample preparation.

Walter Willet, Harvard Medical School
'The role of non genetic factors in chronic disease: no shortage of space for metabolomics'

For the last decade the focus of nutritional advice has been to reduce the total fat intake and consume large amounts of

carbohydrate. However, this advice is inconsistent with many lines of evidence indicating that unsaturated fats have beneficial metabolic effects and reduce risk of coronary heart disease. More recent evidence has also shown that large amounts of carbohydrates consisting of refined starches and sugar, have adverse metabolic effects and increase risks of heart disease and type 2 diabtetes. Yet, many issues remain about the optimal intake of specific types of dietary fats, carbohydrates, and protein. Can metabolomics help resolve these important public health issues?

Bruce Kristal, Harvard Medical School
'Validated biomarkers of caloric restriction in rats: Markers of disease risk in humans?'

Caloric restriction is the most potent and reproducible known means of increasing longevity and reducing morbidity in mammals. This data is directly analogous to human studies linking obesity with poor health outcomes. We are testing whether biomarkers of diet in rats will predict disease in humans. Metabolomic profiles were identified and validated that distinguish caloric intake in rats. These profiles were adapted for human studies, analytically validated at both the instrumentation and at the sample collection levels, then biologically validated. We will present these data and discuss the potential for moving these markers to epidemiological studies in human sera.

Ron Krauss, Children's Hospital Oakland
'Metabolomic Analysis of Variation in Response to Statin Treatment'

Statins, or HMG CoA reductase inhibitors, are widely used for reducing risk of cardiovascular disease (CVD) by lowering LDL cholesterol. However, the response to treatment varies greatly among individuals, and the majority of treated patients remain at risk for CVD. Using samples from a subset of simvastatin-treated participants in the NIH-funded PARC study (Pharmacogenomics and Risk of Cardiovascular Disease) we are testing whether measurements of a panel of plasma lipid metabolites encompassing pathways in sterol and bile acid metabolism can identify interindividual differences in baseline levels and/or statin-induced change that may underlie variation in statin efficacy and may yield information regarding pathways regulating cholesterol metabolism.

Robert Gerszten, Massachussetts Gen. Hosp.
'Towards a Metabolomics Platform for Cardiovascular Disease: Insights from planned Heart Attacks'

Recent advances in analytical chemistry coupled with computational power have enabled a more global approach to metabolite analysis. A benefit of metabolite profiling is that small molecules are closely linked to cellular and whole-body phenotypes, downstream of transcriptional and post-translational modifications, thus providing "proximal reports" of physiological states. Metabolic profiling is particularly relevant to human conditions such as myocardial ischemia and diabetes. Here we report our findings from perturbational studies in humans that highlight potential biomarkers and targets for therapeutic intervention.

Steven Hersch, MIND Institute, Massachussetts Gen. Hosp.
'Huntington's disease: a central neurodegenerative disorder reflected in the peripheral metabolome'

Huntington's disease (HD) is an autosomal dominant progressive fatal neurodegenerative disorder caused by the expression of the huntingtin protein containing an expanded polyglutamine tract. The precise manner in which huntingtin is toxic to neurons is uncertain. We have performed metabolomic analyses using plasma of subjects with HD, including subjects not yet displaying symptoms and subjects with a range of symptom severity. Mutant huntingtin markedly affects the plasma metabolome driven by small molecules that are beginning to be elucidated and which provide insight into pathogenesis as well as potential disease state, progression, and pharmacodynamic biomarkers. More expansive studies are underway.

Rima Kaddurah-Daouk, Duke U
'Metabolomics of Neuropsychiatric Disorders'

Millions of people suffer from mental illnesses or neurodegenerative diseases such as Parkinson's disease, Alzheimer's Disease, schizophrenia, depression, addiction, autism, dyslexia, learning disabilities, among other diseases that all are in much need for better treatments. Unfortunately, our current understanding of these disorders is limited, and by the time neurodegenerative diseases are diagnosed significant number of neurons would have already been lost. There is still a large unmet need for more effective therapies that might slow and possibly halt disease progression. Although some progress has been made in the treatment of psychiatric disorders many patients remain resistant to current therapies. Treatment response and tolerability to antipsychotics or antidepressants varies greatly from patient to patient.

This inherent variability, coupled with intrinsic differences in pharmacology underlie our current inability to predict for the individual patient how he/she may respond to selected therapy. Such uncertainty is distressing for patients and families who engage repeatedly in 'trial and error' choices in search of "the right fit" and for clinicians thus resorting to widespread switching of medications. We have used sophisticated metabolomics analytical platforms and informatics tools to define initial metabolic signatures for several central nervous system (CNS) disorders and for response to drugs used to treat these disorders. We will share early findings from the study of schizophrenia, depression, addiction and Alzheimer's disease.

INVITED SPEAKERS

Steve Bryant, Natl. Center for Biotechnology Information
'PubChem, A Public Repository for Chemical Biology Screening Results'

Presentation Abstract: PubChem provides databases of chemical structures and screening results testing compounds for biological activity. This content is derived from records submitted by both academic scientists and commercial organizations, including chemical structures and/or the readouts of biological activity experiments. In the talk I'll provide an overview of PubChem contents to date and an overview of data analysis tools provided, in particular tools for identifying related biological activity screens and evaluation of structure-activity relationships.

Robert Mistrik, HighChem, Slovakia
'Integrated Approach for Structural Analysis of Endogenous Metabolites'

The challenge set by metabolite profiling is the analysis of numerous small molecules and ends with structural assignments of individual metabolic components. The detailed identification of endogenous metabolites frequently presents a serious bottleneck for converting experimental data into biologically relevant information. A novel integrated mass spectrometric approach which takes advantage of the structural continuum and conservation of eukaryotic metabolism will be presented to address this challenge. Comprehensive empirical data collections in addition to a fragment search technique and precursor-ion fingerprinting method (PIF) are integrated into this approach.

Antonio Vidal-Puig, U Cambridge, UK
'Lipotoxicity, adipose tissue expandability and insulin resistance'

The mechanisms linking obesity to diabetes are not well defined. We and others have developed the adipose tissue expandability hypothesis. Fundamentally the adipose tissue expandability hypothesis states that the absolute amount of lipid an individual stores is less relevant to their susceptibility to metabolic disease, rather it is their capacity to make new adipose tissue that is crucial. When there is a mismatch between energy availability and storage capacity in adipose tissue, this leads to ectopic deposition of lipid in other tissues, including liver and muscle, causing insulin resistance.

Matej Oresic, VTT, Finland
'Metabolic stress, autoimmunity, and diabetes'

Many lines of evidence indicate that early life events play a powerful role in influencing later susceptibility to many chronic diseases. Changes in the concentrations of metabolites during early development, reflecting both the genetic and the environmental factors, may thus aid studies of early disease pathogenesis during the asymptomatic period as well as serve as early biomarkers. We have been conducting an extensive metabolic profiling in a birth cohort of over 8000 children at moderate or high HLA-associated type 1 diabetes risk. We found that autoimmunity may be a relatively late response to early metabolic disturbances.

Arthur L. Castle, Ph.D. Director, Metabolomics and Informatics Program
'The NIH roadmap to understand biological pathways and networks by metabolomics'

The NIH Roadmap was created in 2004 to support transforming research that cuts across traditional Institute missions. Development and implementations of new technologies including metabolomics to better understand biological pathways and networks was one of the major themes of the roadmap. NIH has funded multiple investigators to develop and improve metabolomics technologies and more recently to use these technologies to further hypothesis driven research. An opportunity for funding continues across the NIH within the mission focus of Institutes and Centers. NIDDK has specific interests in understanding the mechanism of obesity, diabetes and metabolic diseases.

Henri Brunengraber, Case Western U
'4-Phosphoacyl-CoAs, a new class of acyl-CoAs'

We investigated the liver and brain metabolism of two drugs of abuse gamma-hydroxybutyrate (GHB) and gamma-hydroxypentanoate (GHP) using a combination of metabolomics and mass isotopomer analysis. We found that these compounds form 4-phospho-acyl-CoAs, a new class of CoA esters. Other 4-hydroxy-acids (up to C_{11}) form 4-phospho-acyl-CoAs. With the C_4 to C_8 4-hydroxy-acids, the formation of 4-hydroxy-acyl-CoAs and 4-phospho-acyl-CoAs resulted in substantial trapping of CoA. Enzyme activity for the activation of GHB and GHP to CoA esters in present in liver cytosol, microsomes and mitochondria. Enzyme activity for the phosphorylation of GHB-CoA or GHP-CoA is present only in the cytosol.

INVITED SPEAKERS

Gert Desmet, Vrije U, Brussels, Belgium

'Optimizing column selection, pressure and temperature for maximal peak capacity in metabolomics'

One of the keys to success in the metabolomics research is the possibility to identify as many possible components in mixtures containing several thousands of different components. Nowadays, the manufacturers of chromatographic columns and instruments offer a panoply of different solutions (use of high temperature and or ultra-high pressures, use of small or large particle size, use of monolithic columns versus packed bed columns,…), all promising to offer the best trail to a maximal peak capacity., In the present contribution, a number of simple guidelines will be given allowing to select the right solution among the different available alternatives.

Chair: Ina Schuppe-Koistinen, Astra Zeneca

'Metabolic profiling in drug R&D: Opportunities for biomarker discovery and personalized healthcare'

Biomarker development forms one of the corner stones of a new working paradigm in Pharma by increasing the practice of linking diagnostic technologies with the use of drugs. Industry and regulators are full of expectations that biomarkers are crucial to generate safe and efficacious drugs; and, essential for deciding what patients should receive which treatment. Profiling of endogenous metabolites offers a major opportunity to systematically identify sensitive and specific plasma or urine biomarkers that could serve as an index of drug action or damage specific to each of the important internal tissues and organs. Although it is technically feasible (and conceptually straight –forward) to identify tissue-specific markers through the use of this technology, often the time required to characterize and qualify new biomarkers is too long to support project decisions. The challenge therefore is to identify relevant biomarkers early enough to implement them for "go, no-go" decisions at critical stages in drug development.

Bruce Hammock, UC Davis

'Case study: The soluble epoxide hydrolase inhibitors as anti-inflammatory agents'

Both global metabolomics and pathway selective metabolomics have proven useful in the development of pharmaceuticals in many ways. These techniques can of course highlight potential risks associated with a compound or target as well as lead to understanding of mechanism. Metabolomic approaches can give a quantitative indication of target engagement which is valuable in development. These assays can be translated into biomarkers of efficacy as single compounds or patterns. However, in an academic laboratory where the budgets are relatively small and funding for moving compounds through target validation and toward investigational new drug status is not encouraged by study sessions, one has to consider the cost benefit of a metabolomics approach. In this presentation one case study will be presented where a metabolomic approach was central in moving a compound into clinical trials and where a metabolomic approach not only supported a biological mechanism of action of the drug in treating hypertension but also opened the possibility that the target could be used to treat inflammation, synergize with NSAIDs, and treat pain as well.

Epoxides of arachidonic acid (EETs) and other biologically active fatty acid epoxides are major contributors to the Endothelium Derived Hyperpolarizing Factor (EDHF) which reduces blood pressure via relaxation of vascular smooth muscle. EETs are rapidly degraded by the soluble epoxide hydrolase (sEH) to the corresponding less active diols. Thus inhibitors of the sEH (sEHI) should increase EET levels leading to the reduction of hypertension through the large-conductance Ca^{++} activated K^{+} channels. Potent transition state mimic inhibitors of the sEH were developed for the recombinant murine, rat and human enzymes then modified to give good ADME. These orally available materials reduced blood pressure in angiotensin driven and other hypertensive models. To confirm target engagement blood levels of lipid epoxides and their corresponding diols were monitored and found to correlate with blood drug levels. However, this laboratory had developed a technology for monitoring a wide variety of bioactive lipids by LC-MS/MS. Surprisingly the effect of sEHI on reducing inflammatory eicosanoids such as PGE_2 in LPS treated animals was even greater than on increasing epoxide to diol ratios. We found that in this model increased EETs transcriptionally down regulated a variety of enzymes associated with propagating inflammation such as induced Cox2 and Lox5. This and other changes results in shifting the pattern of metabolites in the arachidonate cascade from propagation of inflammation toward its resolution. This suggested that sEHI should synergize strongly with NSAIDs which inhibit Cox and with Lox5 and FLAP inhibitors. This hypothesis was verified by metabolomic analysis. These data suggested that sEHI should reduce inflammatory pain which proved to be true. As a control we used several models of neuropathic pain only to find that sEHI were unexpectedly effective. Thus with sEHI metabolomics has proven critical for establishing the expected mechanism and in finding unexpected indications. The epoxide to diol ratio as well as other eicosanoid metabolite measurements are likely to prove valuable biomarkers as the compounds move into human trials.

INVITED SPEAKERS

Vamsi Mootha, Harvard Medical School

'Mitochondrial parts, pathways and pathogenesis'

Mitochondria are central to energy metabolism, apoptosis, and cellular signaling. They contain their own genome that encodes 13 proteins, while the remaining 1000+ proteins are encoded in the nucleus and imported into the organelle. Nuclear gene mutations are responsible for the majority of both rare and common human mitochondrial disorders. Our long-term goal is to use experimental and computational approaches to achieve a complete, mechanistic understanding of the role of the nuclear genome in mitochondrial metabolism and pathogenesis. In this talk, I will present recent work blending genomics, proteomics, and metabolomics, aimed at characterizing the composition, function, and pathogenesis of this organelle.

Minoru Kanehisa, KEGG, JP

'Bioinformatic approaches to integrated analysis of genomic & chemical information'

KEGG (http://www.genome.jp/kegg/) is a biological systems database linking genomic and molecular-level information to higher-level functional information. In addition to KEGG pathway mapping and BRITE hierarchy mapping for biological interpretation of large-scale data sets, we are now proposing chemical structure mapping, based on our recent research projects integrating genomics and chemistry. The basic idea is to link the repertoire of genes in the genome or the transcriptome to the diversity of chemical structures for specific classes of endogenous chemical substances, such as glycans, lipids, and plant secondary metabolites. I will present our comprehensive analysis of glycosyltransferases as a specific example.

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Speaker Abstracts

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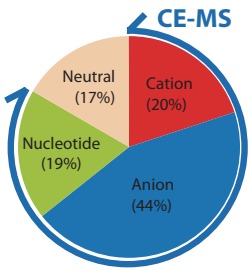
- The most appropriate way for ionic metabolites
- Wide range of applications including
  organs/serum [1], bacteria [2,3], plants [4] and more !



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ORAL ABSTRACT SPEAKERS

Andrew Lane J G Brown Cancer Centre, U Louisville
'The different uses of glucose and glutamine in cancer cells'

Cancer cells are characterized by accelerated aerobic glycolysis („Warburg Effect") and enhanced uptake and utilization of glutamine. The precise metabolic roles of these two major sources in cancer are unclear. We have used stable isotope tracing by NMR from both C-13 glucose and C-13/N-15 glutamine in several transformed cells as well as primary epithelial cells to determine the rates of uptake and the subsequent fate of carbon (and nitrogen for Gln). A substantial fraction of glucose carbon is secreted as lactate, wheras glutamine carbon enters mainly glutamine, GSH, pyrimidine rings and protein.

Facundo Fernandez, Georgia Tech U
'Ovarian cancer serum metabolomics by LC/TOF MS and direct analysis in real time (DART) / TOF MS'

In this presentation we describe baseline metabolomic studies conducted on human sera focusing on recognizing "panels" of potentially-diagnostic ovarian cancer biomarkers. Using LC/TOF MS, we analyzed serum samples from 37 ovarian cancer patients and 35 controls. Genetic algorithms coupled to partial least squares discriminant analysis were applied to the MS data. Multivariate models were examined under full crossvalidation conditions. The best positive and negative ion mode electrospray ionization models showed sensitivities of 97.2% and 100.0%, and specificities of 100.0% and 100.0%, respectively. Higher-throughput serum metabolome profiling tools based on Direct Analysis in Real Time (DART) MS being developed by our group will also be introduced.

Jeff Schuster, Metabolon
'Investigation of prostate cancer aggressivity using metabolomics'

Prostate cancer is diagnosed in >200,000 men each year. It is very difficult to determine which cancers are indolent and which are aggressive and may metastasized. Tests that distinguish indolent from aggressive tumors have potential to reduce the number of unnecessary biopsies and prostatectomies. Prostate cancer (PCa) aggressivity was investigated at the biochemical level using metabolomics. Studies with pre-biopsy patient urine and post-surgical tissues identified compounds changed between controls, localized, and metastatic PCa. Subsets of PCa aggressivity biomarkers were observed in both urine and tissues. These biomarkers are excellent candidates for development of diagnostic tests for prostate cancer aggressivity.

Teresa Fan, U Louisville
'Altered Regulation of Metabolic Pathways in Human Lung Cancer Discerned by 13C-Isotopomer Profiling'

Metabolic perturbations arising from malignant transformation have not been systematically characterized in human lung cancers in situ. Metabolomic analysis allows genome-wide functional analysis of the dysregulation of cancer-specific genes. Metabolic changes were investigated by infusing uniformly labeled 13C-glucose into human lung cancer patients, followed by resection and processing of paired normal lung and tumor tissues. 13C-isotopomer-based metabolomic analysis was performed using NMR, GC-MS, and MS-filtered FT-ICR-MS. Many polar metabolites were consistently found at higher levels in tumor tissues than their normal counterparts. As expected, the 13C-isotopomer analysis indicated activated glycolysis in the tumor tissues. In addition, the Krebs cycle activity was altered. This was evidenced by an enhanced buildup of 13C-succinate in tumor tissues, consistent with the pathway from glucose to succinate via glycolysis, anaplerotic pyruvate carboxylation (PC), and the reverse Krebs cycle sequence from oxaloacetate to succinate. PC activation in tumor tissues was also supported by the increased expression of pyruvate carboxylase transcripts. PC activation – revealed here for the first time in human patients – is likely necessary to replenish the Krebs cycle intermediates to fulfill the high anabolic demands for growth in lung tumor tissues. We hypothesize that this is an important dysregulatory event in lung and possibly other tumor development.

FT-ICR-MS analysis of lipid extracts of paired normal and cancerous lung tissues as well as plasma of lung cancer patients and normal volunteers was also performed. Thousands of automated assignment on phospholipids and their 13C-isotopomers were made by employing "PREMISE" (Precalculated Exact Mass Isotopomer Search Engine) software developed in-house. Preliminary comparison of the assigned lipids indicates a fast turnover of plasma phospholipids in lung cancer patients. Further comparison is being conducted to reveal potential lipid marker(s) for lung cancer.

WN Paul Lee, UC Los Angeles
'Amino acid synthesis in HepG2 cells: insight from isotopomer analysis'

Amino acid biosynthesis system in mammalian cells is intricately coupled to that of glucose metabolism. It depends on the nutrient environment provided by the culture medium, compartmentalization of the pathways and the metabolic phenotype of the cell. The utilization of glucose by the HepG2 cell reflects the optimization of glucose for membrane lipids, nucleic acids and protein synthesis and the function of the metabolic network within the cell or its metabolic phenotype. Our study using [U13C6]-glucose shows that amino acid synthesis is compartmentalized and is very active in mammalian cells in culture.

Johanna Scarino, Princeton

'Active metabolism in fibroblasts after exiting the cell cycle'

Many of the cells in the human body spend large spans of time in the non-proliferating state known as quiescence. Understanding quiescence is thus critical to understanding how normal cells function within the human body. Currently, the differences between the quiescent state and the proliferative state are being investigated in cultured primary human fibroblasts using a metabolomics approach. Through the use of liquid chromatography mass spectrometry and isotopically labeled nutrients we have been able to monitor both expected and unexpected differences and similarities between the metabolisms of these two cell states.

Gregor McCombie, U Cambridge, UK

'A combined NMR and mass spectrometry metabolomic study of weight loss and omega-3 oil intake determines selective short chain triglyceride reduction'

A large number of studies have shown independent beneficial effects of weight loss and fish oil supplement interventions on a variety of cardiovascular disease risk factors, including dyslipidaemia, raised blood pressure, insulin insensitivity, inflammatory markers and adiponectin concentration. However, it is unknown what metabolite changes occur during these two interventions and whether these changes may mediate some of the beneficial effects. In this study, 93 subjects were randomised to one of two weight loss interventions or a control group. The weight loss groups in addition received either fish oil or placebo oil for the duration of the study. Fasting blood samples were collected at baseline, 12 and 24 weeks. A metabolomic approach using a combination of 1H-Nuclear Magnetic Resonance (NMR), Gas- and liquid chromatography coupled to mass spectrometry was employed to elucidate the changes in blood plasma following the intervention. Initial analysis by NMR spectroscopy demonstrated that the major changes in the metabolome of the blood plasma were associated with lipids and not low molecular weight aqueously soluble metabolites by NMR. Lipid profiles changed dramatically with fish oil and more subtly with weight loss. All three analytical approaches demonstrated that the main changes were due to the degree of saturation of the fatty acids found in the plasma from subjects, in part reflecting the supplemented oils but also their catabolism. Specifically, fish oil supplementation increased the proportion of various phospholipid species. The previously reported reduction in total triacylglycerides (TAGs) with fish oil supplementation (Browning, L.M., et al., Int J Obesity, 2004. 28. 1004), was driven by a specific shorter chain length subset of the measured TAGs. This indicates a specific metabolic perturbation in triglyceride metabolism rather than a global decrease in all triglycerides.

Elin Chorell, Umea, Sweden

'Nutrition-modulation by predictive metabolomics'

A predictive metabolomic investigation of nutrition modulated recovery in humans, following strenuous exercise, is presented. The results provide a detailed map for choosing, monitoring and controlling individual treatments as well as for detecting individuals with different response to treatments, e.g. slow and fast responders.
The study involved 24 male subjects performing 90min of strenuous ergometer cycling followed by an equally long recovery period. This test was repeated four times, and immediately after performed exercise the participants were given a drink consisting of either water or one of three macronutrient drinks (no1: containing 1g carbohydrates, no2: containing 1.5g carbohydrates and no3: containing 1g carbohydrates + 0.5g protein, in relation to the subjects bodyweight in kg). The study was performed according to an organised design so that no bias confounding the variation of interest was introduced into the data. The objective of the present study was to investigate the metabolic response in relation to the ingestion of macronutrients following strenuous exercise. This was reflected by screening-analysis of low molecular weight factors in blood serum using multiple analytical platforms, e.g. GC-TOF/MS, NMR and UPLC-MS. The hyphenated data were processed using hierarchical multivariate curve resolution (HMCR) to generate pure spectral and chromatographic profiles for identification and quantification. Orthogonal partial least squares (OPLS) was used to model the systematic variation related to the macronutrient effects. Extensive validation was carried out including predictive HMCR, sevenfold full cross-validation and OPLS predictions. In addition, metabolic patterns related to the subject's fitness level were investigated together with the individual response to nutritional modulation in the early recovery phase for subjects defined as unfit. The results did verify that we could detect systematic changes in a large number of identified or identifiable metabolites in

human serum related to different macronutrient intake and fitness status in the early recovery phase following exercise. In addition we could interpret these metabolic interactions, and importantly modulate as well as make predictions of the metabolic status in human on an individual basis.

Christopher Crutchfield, Princeton

'The metabolic response of yeast to nutrient limitation'

WE examined changes in yeast metabolism that occur as a response to limitation of glucose, nitrogen, phosphate, uracil (in a uracil auxotroph) , and leucine (in a leucine auxtroph) in chemostat culture. Assaying the concentrations of intracellular metabolties voa LC-MS/MS analysis has provided a dataset that reveals a fundamental relationship between the nutrient limitation and intracellular metabolome. This approach of examining the steady-state metabolome of nutrient limited yeast, especially when combined (in ongoing work) with genetic perturbations, holds promise for better understanding the interplay of external environment, signaling systems, and metabolism in eukaryotic cells.

Kian-Kai Cheng, U Cambridge, UK

'A metabolomic study of a mouse model of atherosclerosis'

Atherosclerosis is a process of progressive arterial wall thickening and is the main cause of cardiovascular disease. A number of mouse models have been developed to investigate this pathology including the apolipoprotein E gene knockout mouse, the low density lipoprotein receptor knockout (LDLR-/-) mouse, and the wild type C57BL/6 mouse which is susceptible to diet-induced atherosclerosis. In this study, we have used a factorial design with two genotypes (C57Bl/6 and LDLR-/- mice) and diets (control RM1 and high fat diet) in a twelve-week experiment. All animals were then fed a control RM1 diet for another six weeks to examine the return to normality for both mouse strains. Urine and blood plasma were collected during the study (week 0, 4, 8, 12 for urine; 8, 12 & 18 weeks for blood plasma) and analyzed by 1H NMR spectroscopy at 500 MHz. Plasma samples were also biochemically assayed for triglyceride and cholesterol ester content. In addition, atherosclerotic plaque development in inominate arteries was quantified by three-dimensional fast spin-echo magnetic resonance imaging (MRI). The NMR based metabolomic dataset was examined by a combination of ANOVA, multivariate statistics and a novel technique called Pilot PCA in order to study the effect of the main factors (i.e. diet and genotype), interaction of factors, and the correlation between metabolite changes and the clinical data. When fed on the high fat diet, both C57BL/6 and LDLR-/- mice had an increased concentration of betaine and dimethylglycine in plasma, as well as decreased excretion of citrate, alpha-ketoglutarate, succinate, hippurate, and taurine in urine. The urinary betaine concentration was significantly higher in the C57BL/6 mice on high fat diet as compared with other groups. This indicated an interaction effect of both diet and genotype on urinary betaine excretion. The high fat diet in the LDLR-/- mice caused a ten-fold increase in plasma cholesterol which returned to a normal concentration at week 18 following the switch to the RM1 diet. In addition, a significant increase in the MRI lesion area was observed in the LDLR-/- mice on the high fat diet ($p<0.001$). This group of animals was also discriminated from the three other groups by markedly high concentrations of plasma betaine to urinary betaine ratio ($p<0.001$). This study suggests that metabolites in urine and blood plasma could be used to monitor atherosclerosis onset and drug efficacy during treatment.

Christine Des Rosiers, U Montreal, Canada

'Isotopomer analysis as applied to the heart: From metabolic flux analysis to therapeutic strategy'

Similar to metabolomics, metabolic flux analysis can provide novel and unexpected insights into the phenotype of normal and diseased hearts – a point illustrated by a recent study conducted in the mdx mouse, a model of dystrophic cardiomyopathy. Specifically, working mdx mouse hearts displayed a metabolic shift from fatty acid towards carbohydrate oxidation to acetyl-CoA formation for energy production and a significant decrease in mitochondrial citric acid cycle pool size; changes associated with exacerbated oxygen consumption as well as compromised function and sarcolemmal integrity. The documentation of the latter pathophysiological changes highlights the power inherent in measuring metabolic fluxes as one component in predicting the physiological phenotype of any organism. In this regard, over the past 10 years, a gas chromatographic-mass spectrometric-based stable isotope (isotopomer)-based approach was developed and applied to probe the myocardial metabolic phenotype. This approach involves ex vivo perfusion of working rat and mice hearts with substrate mixtures labeled with carbon 13 and allows for detailed and simultaneous determinations of various hemodynamic and metabolic flux parameters relevant to energy production. This approach enabled us to document that the metabolic alterations of the mdx heart preceded the development of overt cardiomyopathy, suggesting that they may represent an early "subclinical" signature of defective nitric oxide and/or cGMP signaling. Subsequent testing for this cGMP signaling defect using transgenic and pharmacological approaches demonstrated improvement of contractile performance, myocardial metabolic status, and sarcolemmal integrity, thus suggesting a potential clinical avenue for the treatment

of the dystrophin-related cardiomyopathies. Collectively, results obtained using the ex vivo working mdx mouse perfused with carbon 13 labeled substrates illustrate how changes in myocardial metabolic fluxes may constitute a sub-clinical signature of cell signaling occurring prior to overt cardiomyopathy as well as provide a basis for therapeutic strategy.

Hans-Peter Deigner, Biocrates, Austria

'Targeted Metabolomics on Age-dependent and Stroke-induced Lipids'

We are investigating age-related changes in gene expression and metabolism and aim at relating these changes to stroke-induced consequences. Age-related alterations in the brain involve abnormalities in ceramide and cholesterol metabolism. Recent advances in lipid biology research have also demonstrated that neuronal sphingolipids are essential for brain development. Within a stroke project, methods with clinical utility are being developed. First exciting results of experiments addressing stroke-induced and age-related effects by targeted metabolomics reveal changes of lipid patterns. For instance, a significant age-dependent difference of desmotherol concentration is detectable in brain lipids, isolated from 2 and 9 month old animals.

William Wikoff, Scripps

'Using metabolomics of animal models to understand complex human biochemistry and disease'

Human diseases, even those which arise from single point mutations, typically manifest in complex downstream effects, involving multiple organ systems and thus potentially affecting multiple biochemical pathways. Animal models provide an experimental system which reflect the level of complexity found in human biochemistry and disease. We have studied two very different animal models of viral infection: a primate model of neuroAIDS, and a mouse model of viral immunology. In the neuroAIDS model, rhesus macaques are infected with SIV, eventually develop encephalitis and die. Cerebrospinal fluid is used for metabolomics because it reflects the biochemical state of the brain, and is an accessible biolfluid in humans. The second system is LCMV infection in mouse, which produces a rapid immune response in a well-defined time frame.

A global metabolomics approach was used to study the neurochemical effect of SIV infection in rhesus macaques, a model system for HIV and neuro AIDS, and illustrates the potential of metabolomics to address problems in central nervous system biochemistry and neurovirology, as well as neurodegenerative diseases. Cerebrospinal fluid (CSF) was compared before and after viral infection, and more than 3,500 features were measured. There were significant changes in the metabolome, with a general increase in metabolite concentrations during infection. Specific metabolites which changed were identified using database searching and comparison of the MS/MS pattern using a QTOF. Fatty acids, including palmitic, myristic, oleic, and stearic acids increased during infection, as did the corresponding lysophosphocholines. Other molecules that increased significantly during infection included include carnitine, and the acylcarnitines, octanoylcarnitine and butyrylcarnitine. All of these molecules are related to fatty acid and lipid metabolism. Gene chip experiments were then performed on the hippocampus of uninfected compared to infected animals. These results indicated that different phospholipase genes, including PLA1 and PLA2 were up-regulated during infection. This increase in transcript levels was confirmed using quantitative PCR. Finally, specific biochemical assays were performed on brain tissue, indicating that phospholipase 2 activity was indeed increased in the brain as a result viral encephalitis. Thus, the metabolomics results were able to generate a testable hypothesis, which was confirmed by using gene chip experiments and biochemistry. These three datasets reinforce each other, and demonstrate the process of going from descriptive metabolomic phenomenology to a testable biochemical hypothesis, even in a disease as complex as neuroAIDS.

The second system involves a study of renal transport. In the kidney, molecules are transported specifically by membrane proteins in the proximal tubules of the nephron. A system of organic acid transporters (OAT's) carries organic anions such as para-aminohippurate and may be involved with either secretion or reabsorbtion. Much of the interest in these systems has arisen because these proteins are responsible for the transport of many drugs, and are therefore closely tied to issues of drug clearance and toxicity. However, these transporters clearly did not evolve for the purpose of transporting drugs through the kidney, and the transport of endogenous compounds is of central importance, particularly because most of the major "uremic toxins" are known to be transported by one or more of these OAT's. By examining knockouts of individual OAT's, and applying an untargeted metabolomics approach to both plasma and urine, we have been able to determine what endogenous molecular substrates of these transporters. In the case of one transporter, a potential link was found between the reduced blood pressure phenotype and the molecular substrates identified by metabolomics.

The combination of metabolomics, transcriptomics and biochemistry, greatly facilitates the process of understanding metabolomic changes in a biological context. Other specific approaches can be used, such as immunohistochemistry, to localize changes at tissue and cellular resolution, or to determine substrate binding and kinetics in and ex vivo model, as was done with the kidney transporters. The use of metabolomics to understand and

actually solve complex biomedical and biochemical problems using animal models will be emphasized. The integration of various techniques with metabolomics to understand these complex systems will be emphasized.

Marlon Quinones, UNC Chapel Hill

'Exploring the impact of lithium treatment on the metabolome: A global biochemical dissection of treatment-response correlates in bipolar disorder'

Background

Metabolomic studies capture global biochemical events by assaying thousands of small molecules in cells, tissues, organs, or biological fluids, followed by the application of informatics techniques to define metabolomic signatures. Here in the first study of its kind, we use the power of metabolomics to define biochemical effects of Lithium and identify potential correlates of treatment response in bipolar disorder.

Methods

Individuals with DSM-IV Bipolar Disorder Type I (n=11, age= 27±4.7yo, 80% females and medication-free for at least 2weeks) experiencing a depressive or mixed episode received daily treatment with Lithium for 4weeks. During this time, the dose of Lithium was tailored to reach therapeutic blood levels between 8-1.2 mEq/L. Trained raters assessed mood symptoms before and after treatment using the Young Mania Rating Scale (YMRS) and Hamilton Depression Rating Scale (HAMD). Response was defined as a 50% reduction in mood ratings. The magnitude of responses was defined as percentage reduction in mood ratings. Blood samples were collected at the time of mood evaluation, plasma isolated immediately and frozen. Biochemical assessment was conducted using GC-TOF mass spectrometry in conjunction with BinBase data annotation. 246 metabolite signals were reported of which 89 were identified with chemical structures. Principal Components Analysis (PCA) and Partial Least Square (PLS) analysis were conducted. For ANCOVA (pre- vs. post-treatment) and Partial correlations, age and sex were used as covariates (significance= uncorrected $P<0.05$). Correction for multiple comparisons was conducted using False Discovery Rate (FDR; corrected *P, cP*).

Results

Lithium treatment was associated with significant reductions in depression (17.8±6.4 vs. 5.2±3.2, $P=0.000003$) and mania (11.4±8.7 vs. 3.3±3.5, $P=0.003$) rating scores. All the subjects responded to treatment. Based on 89 metabolites that were identified in plasma samples, a clear separation between pre- and post-treatment samples was seen on PCA and PLS plots. Treatment was associated with a significant change in levels of 20 metabolites (uncorrected $P<0.05$). Some of these metabolites included molecules belonging to pathways postulated to be responsible for Lithium's therapeutic effects such as Inositol (55760±10736 vs. 43755±9733, *P= 0.01*), Arachidic Acid (5580±1294.55 vs. 4266±1242, *P=0.04*), Tryptophan (214823±37736 vs. 172756±39449, *P=0.02*) and Glutamine (619704±110174 vs. 488743±82088, *P=0.002*). Only, changes in Glycerol, an intermediate in carbohydrate and lipid metabolism, whose levels were previously described to be affected by Lithium; and being abnormal in Depression, remained significant after FDR correction (304387±107376 vs. 169816±46938, $cP=0.0009$). Of note, while Lithium-induced reduction on depressive symptoms was significantly correlated with pre-treatment levels of gamma-tocopherol ($r=-0.81$, $P=0.004$) and Octadecanol (a fatty alcohol; $r=0.82$, $P=0.006$) reduction on manic symptoms was correlated with levels of Urea ($r=0.94$, $P=0.0001$).

Conclusions

Global dissection of changes induced in the metabolome by Lithium might provide the opportunity to identify clinically relevant biomarkers of treatment response. Replication studies are warranted to determine whether levels of antioxidants and Urea might serve predictors of response to Lithium. Gamma-tocopherol (an isoform of Vitamin E) might play a critical role in

Reza M Salek, U Cambridge, UK

'A metabolomic comparison of mouse models of Neuronal Ceroid Lipofuscinoses'

The Neuronal Ceroid Lipofuscinoses (NCL) are a group of fatal inherited neurodegenerative diseases in humans distinguished by a common clinical pathology, characteristic storage body accumulation in cells and gross brain atrophy. We have investigated metabolic changes in 2 mouse models of NCL to examine metabolic profile of each model and compare to the existing Cln3 (Batten disease) mouse model [1]. The first model, a naturally occurring mouse mutant, termed motor neuron degeneration (mnd) mouse contains a one base pair insertion in the orthologous mouse Cln8 gene (82% identical to human gene), on a C57BL/6J background, exhibiting abnormalities akin to those in human NCL patients. The second mouse variant models late infantile NCL and is termed the neuronal ceroid lipofuscinosis (nclf) mouse. The nclf mouse contains a one base pair insertion in the orthologous mouse Cln6 gene (90 % identical to human gene) resulting in a frameshift defect. Similar to mnd mice, nclf mice on a C57BL/6J background, exhibit a characteristic accumulation of autofluorescent lipopigment in neuronal and non neuronal cells.

Brain tissues from mnd, nclf, and C57BL/6J controls, of various ages (1, 2, and 6 months) were studied using a chloroform/methanol extraction method prior to analysis by 1H NMR spectroscopy. All the metabolites were assigned using the existing literature, 2D NMR spectroscopy assisted by STOCSY analysis [2]. The NMR profiles derived from mnd and nclf mice were distinguished according to disease/wild type status in the cortex, cerebellum. In particular, glutamine was increased and gamma-amino butyric acid (GABA) decreased in the cortices of mnd (adolescent mice) and nclf mice relative to wild type in all ages of mice examined. In younger mice phosphocholine and myo-inositol were increased in the cortex of mnd mice and N-acetyl-L-aspartate (NAA) concentration was decreased; in nclf mice the contrary was observed. Comparison of our result to the existing Cln3 mice model (with similar ages ranges) showed that the metabolism of mnd mice resembled older Cln3 mice, where the disease is relatively advanced, while the metabolism of nclf mice was more akin to younger Cln3 mice, where the disease is in its early stages of progression. Our results allowed us to identify metabolic traits common to all NCL subtypes. Similar analyses should prove invaluable in understanding more of the underlying disease pathogenesis.

1. Pears, M.R et al., High resolution ^{1}H NMR-based metabolomics indicates a neurotransmitter cycling deficit in cerebral tissue from a mouse model of Batten disease. J Biol Chem, 2005. 280(52): p. 42508-14.

2. Cloarec O. et al, (2005) Evaluation of the orthogonal projection on latent structure model limitations caused by chemical shift variability and improved visualization of biomarker changes in ^{1}H NMR spectroscopic metabonomic studies. Anal Chem 77, 517-526.

Tobias Kind, UC Davis Genome Center

'Annotation of unknown metabolites with accurate mass LC-MS/MS and GC-MS by constraining metabolite database queries using analytical metadata'

Metabolic profiling experiments using gas chromatography and liquid chromatography coupled to mass spectrometers (GC-MS, LC-MS) routinely detect 300 components. However, less than one third of these peaks are routinely identified. Unfortunately, the diversity of metabolism and the availability of pure reference compounds render it unlikely that we will ever have fully comprehensive spectral libraries. We propose to apply algorithms that utilize all available chromatographic and mass spectrometric information to rank potential metabolite annotations from matching experimental and calculated structure properties. Metabolic signals that are annotated by high scores would then complement those metabolites that are truly identified by authentic standards.

Philip Britz-McKibbin, Assistant Professor, McMaster University

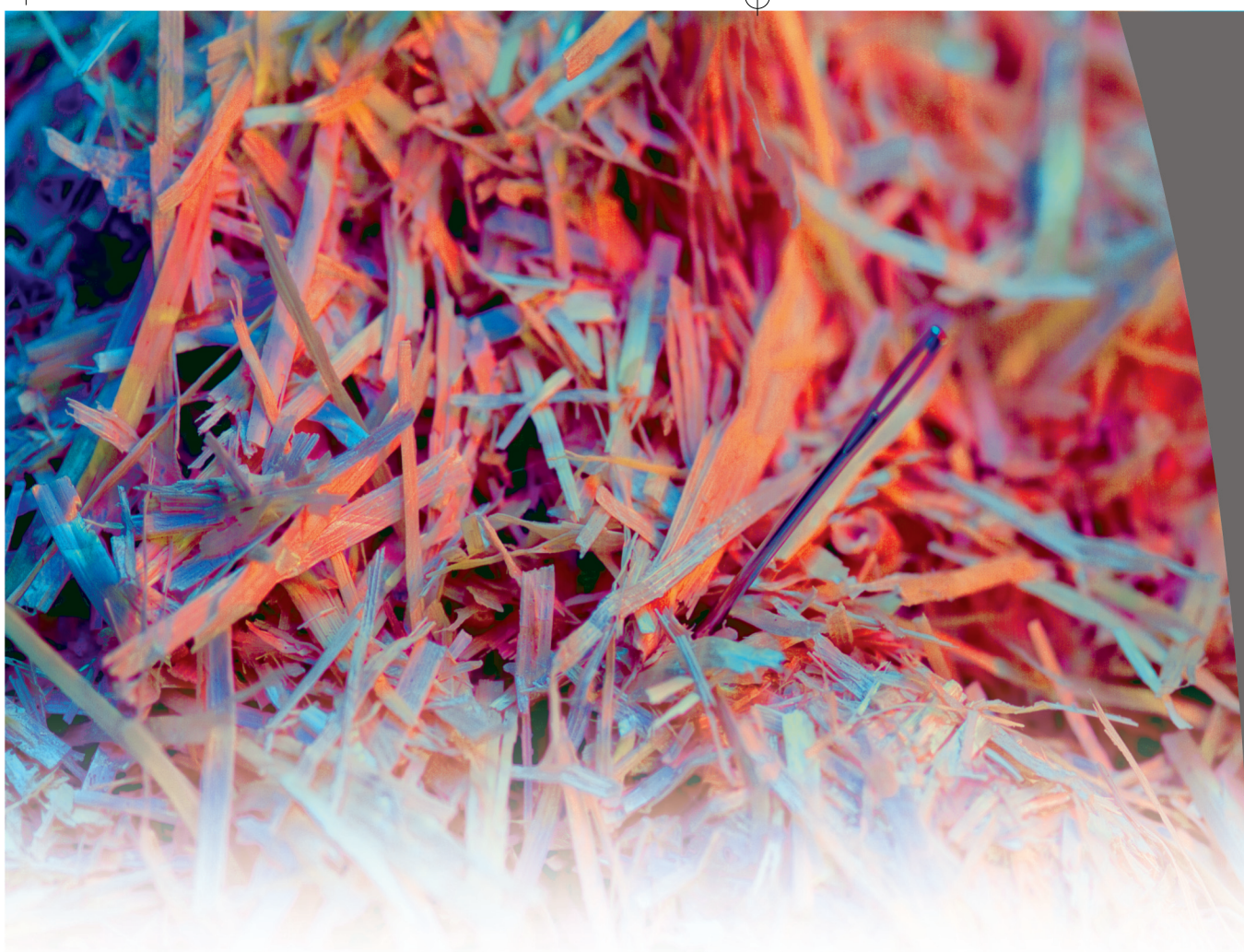
'Virtual Metabolomics: Metabolite Identification and Quantification Without Chemical Standards'

A major hindrance in metabolomics research is the presence of a large fraction of unknown metabolites detected in complex biological samples when purified chemical standards are unavailable and metabolite databases remain incomplete. Herein, we demonstrate an integrative strategy for *de novo* identification and quantification of metabolites using capillary electrophoresis-electrospray ionization-mass spectrometry based on fundamental electrokinetic, thermodynamic and molecular properties of an ion. Virtual metabolomics offers a promising way to identify and quantify novel biomarkers and unknown drug metabolites *in silico* while minimizing time-consuming chemical synthesis protocols that are required for rapidly expanding metabolomic initiatives.

Lee Roberts, U Cambridge, UK

'Metabolic phenotyping of adipocyte differentiation and peroxisome proliferator activated receptor delta Activation in the 3T3-L1 cell line'

The 3T3-L1 murine cell line is derived from the 3T3 mouse fibroblast cell line and has become a well established model for mammalian adipogenesis. In the preconfluent state 3T3-L1 cells morphologically and biochemically resemble fibroblasts. However once the cells reach confluency they can be differentiated into mature adipocytes using a combination of hormones. The process is robust and relatively uniform with 80-90% of cells differentiating and mirroring changes observed during mammalian adipocyte development. The differentiation of preadipocyte cells into the mature adipocytes requires a complex interaction of metabolic pathways that as yet remain to be fully defined. Many of these pathways may only be critical to differentiation at very specific points during the process. Using a combined Gas Chromatography-Mass Spectrometry (GC-MS), 1H-Nuclear Magnetic Resonance (NMR), Liquid Chromatography-Mass Spectrometry (LC-MS) and Direct Infusion-Mass Spectrometry (DI-MS) approach to metabolomics, in conjunction with multivariate statistics, the metabolic phenotype of differentiating 3T3-L1 preadipocytes as they mature into adipocyte cells has been investigated. A diverse range of pathways were altered



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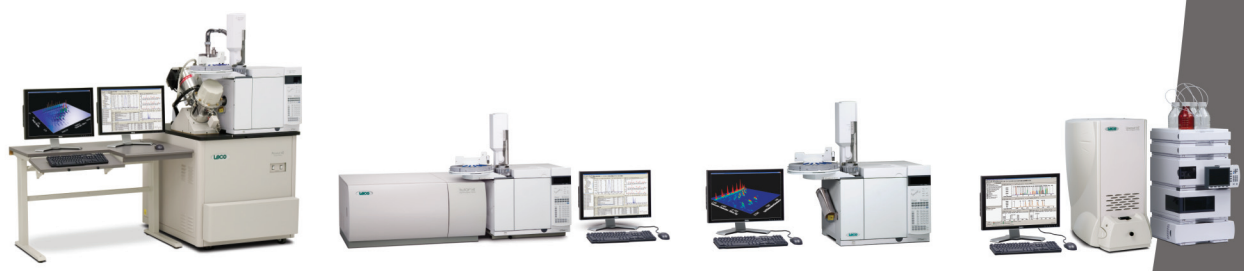

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during the differentiation process including polyamine biosynthesis, essential fatty acid pathways, fatty acid desaturation as well as fatty acid synthesis and the TCA cycle. Lipid accumulation is detected, initially as an increase in the saturated fatty acids followed by increases in the concentrations of the desaturated fatty acids later in the differentiation process. The increase in the concentration of desaturated fatty acids is accompanied by decreases in both palmitate and stearate. Increases in TCA cycle intermediates are detected early in differentiation and again prior to lipid accumulation. Polyamine biosynthesis also increases prior to lipid accumulation before decreasing once terminal differentiation occurs.

In addition the effect of activation of the Peroxisome Proliferator-Activated Receptor (PPAR) delta has been investigated in this cell line. PPAR delta is a ligand-activated transcription factor and member of the nuclear receptor superfamily. It is expressed almost ubiquitously, but in particular high levels of expression are present in adipose tissue, liver and skeletal muscle. Although the physiological ligands of PPAR delta remain to be defined, high affinity synthetic ligands have been developed for the receptor as a therapeutic target for type-2 diabetes mellitus, dyslipidaemia and the metabolic syndrome.

Ville-Petteri Mäkinen, Helsinki U Tech, Finland

'Metabolic characterization of vascular complications and their progression by proton NMR spectroscopy of serum in patients with type 1 diabetes'

Vascular complications are the primary cause of mortality in patients with type 1 diabetes. The goal was to assess whether high risk metabolic phenotypes could be determined by ^{1}H NMR spectroscopy. The NMR experiments were targeted at three molecular windows: i) a standard spectrum of serum, ii) a T2filtered spectrum of low molecular weight metabolites and iii) a spectrum of lipid extracts. The three molecular windows were able to distinguish stable patients from those that progressed to vascular diseases; patients that were clinically the most uncertain (micro-albuminuria) were characterized by the fatty acid composition and other metabolic traits.

Daniel Vis, U Amsterdam, NL

'Dynamic endocrine networks, revealing dynamic differences between health and disease'

Systems biology revolves around connecting small bits and pieces of a puzzle to study the emergent properties of the hidden biological system. This approach is snowballing through the life sciences and opens many new settings for research and hypothesis testing. Systems biology eluded medicine for a long time, though this field is now turning to a more adopting strategy to systems biology in the form of network medicine. Here we show how the systems biology idea can be applied to the endocrine domain. Hormones tend to be secreted in episodic bursts. We developed tools to identify and quantify these bursts, and from detailed 24-hour, 10-minute interval time series data we show that intimate relations between hormones exist. We propose a generalized working scheme to arrive at a hormone association network and show that these associations can vary in strength between healthy and diseased status. Furthermore by co-integration of the hormone and pulse data we aim to arrive at the driving forces behind some hormones. These tools will provide clinical endocrinologists with a new view of the most basic form of information in their field. The used endocrine data is a great starting place for developing dynamic models, like the ones detailed here. In the future we aim to extend the work on dynamic modeling to metabolomics data. This generalized working scheme may allow identification of hubs in dynamic (metabolic) data, thus aiding more targeted approaches in systems where the functions of the metabolites are unknown.

Daniel Amador-Noguez, Princeton

'Metabolome dynamics of the glycolysis/gluconeogenesis switch in Escherichia coli'

The acetate switch in *Escherichia coli* occurs when cells are transferred from glucose to acetate, which requires them to perform gluconeogenesis to fulfill their biosynthetic needs. Using liquid chromatography-tandem mass spectrometry we have studied the dynamic behavior of this system by directly quantifying the levels of metabolites involved in all major pathways of central metabolism. Isotopic tracers have been employed to probe the associated flux changes. Our findings are consistent with the existence of a rapid cellular system for termination of glycolysis, paired with a slow system for activating gluconeogenesis and should inform the general question of regulation of flux direction in reversible metabolic pathways.

Fionnuala Morrish, Fred Hutchison Cancer Center

'Metabolites and post-translational protein modification: how oncogene regulation of the metabolome enables activation of protein signaling networks'

Cellular metabolism provides essential substrates for post-translational modification of proteins. Here we show that c-Myc interconnects substrate supply and acetylation of proteins. Using [U-^{13}C] glucose and a combination of

GC/MS and collision-induced dissociation tandem mass spectrometry, we determined that Myc increased the fractional incorporation of ^{13}C labeled 2-carbon fragments into the fatty acid palmitate, and the acetyl-lysine K16 residue (y5 fragment ion of 4-17 tryptic peptide) of histone H4, in *myc*-/- and *myc*+/+ Rat1A fibroblasts. These data demonstrate a novel connection between Myc's transcriptional regulation of the histone acetylase GCN5, enabling global chromatin remodeling, and Myc's regulation of the metabolome.

Fangping Mu, Los Alamos National Lab

'Carbon-fate maps for metabolic reactions'

Stable isotope labeling of small-molecule metabolites combined with analytic techniques for measuring isotopomer distributions has a number of important applications, including the determination of chemical structures, characterization of enzymatic reaction mechanisms and elucidation of metabolic pathways (1-3). Analysis of isotope labeling patterns requires knowledge of the fates of individual atoms and moieties in reactions, which can be difficult to collect in a useful form when considering a large number of enzymatic reactions. Atom fate maps are not included in commonly used archives of metabolic knowledge, such as the Kyoto Encyclopedia of Genes and Genomes (KEGG), MetaCyc, BRENDA or the biochemical pathways wall chart of Roche Applied Science. We report carbon-fate maps for 4605 enzyme-catalyzed reactions documented in the KEGG database(4). We used a systematic structure-based system for naming and referencing metabolites and their carbon atoms (http://www.iupac.org/inchi/). Every fate map has been manually checked for consistency with known reaction mechanisms. A map includes a standardized structure-based identifier for each reactant (namely, an InChITM string); indices for carbon atoms that are uniquely derived from the metabolite identifiers; structural data, including an identification of homotopic and prochiral carbon atoms; and a bijective map relating the corresponding carbon atoms in substrates and products. A fate map also includes an identification of carbon atoms in reaction centers. A fate map also includes all the information required for display by FateMapViewer, our software tool for visualizing carbon fate maps. Fate maps are defined using the BioNetGenTM language (http://bionetgen.lanl.gov/), a formal model-specification language. As a result of this feature, maps can be automatically interpreted by BioNetGenTM language to obtain mass-balance equations and simulate stationary or dynamical labeling patterns.

Warwick Dunn, U Manchester, UK

'Metabolic alterations are observed in the metabolomes of plasma and placental tissue related to pre-eclampsia'

Pre-eclampsia is a common multi-system disorder that complicates 2-5% of pregnancies and is a leading cause of maternal death, perinatal morbidity and perinatal mortality. Furthermore, infants born in a pre-eclamptic pregnancy are at an increased risk of hypertension, heart disease and diabetes in adult life. The pathogenesis of pre-eclampsia is thought to involve inappropriate adaptation of the interface between the maternal vasculature and the developing placenta early in pregnancy which subsequently leads to the development of a poorly perfused feto-placental unit. In this model, continuing poor perfusion of the placenta is proposed to result in the secretion of a factor(s) into the maternal circulation. These cause 'activation' of the vascular endothelium. The clinical syndrome of pre-eclampsia results from widespread changes in endothelial cell function in both small and large vessels. The authors are performing complementary research strategies to define the metabolic phenotype of pre-eclampsia in plasma and placental tissue. These involve detecting predictive metabolic biomarkers in plasma at mid (pre-symptomatic) and late (symptomatic) stages of pregnancy and investigating the phenotypic differences of healthy placental tissue grown under hypoxic and normoxic conditions. Pre-eclamptic phenotypes are hypothesized to be hypoxic-like. Both strategies will be discussed in this presentation:

(a) description of alterations in the plasma metabolome of pre-eclamptic subjects compared to matched controls in pre-symptomatic and symptomatic stages of pregnancy employing GC-MS and UPLC-MS. A description of appropriate experimental design for large-scale metabolomic investigations and the application of quality control (QC) samples will be discussed and shown to provide results of greater robustness, reproducibility and validity in this study.
(b) metabolic footprinting studies of placenta tissue from normal pregnancies cultured under normoxic (6%) and hypoxic (1%) conditions to assess metabolic differences related to hypoxia. Validation of placental sampling and analysis will also be described.

André Canelas, TU Delft, NL

'Getting the right numbers: how to avoid some (common) mistakes in metabolomics-based research in S. Cerevisiae'

Accurate measurement of intracellular metabolite levels is of prime interest in the study of metabolic reaction networks and their regulation in vivo. Despite recent advances in analytical tools, which led to the advent of the

field of metabolomics, obtaining accurate, reliable data on intracellular metabolite levels is still not straightforward. We shall analyze some of the limitations in yeast metabolomics and describe the approaches we have used to tackle them.

Currently, one of the major constraints in metabolomics of eukaryotes is compartmentation. Direct measurements provide whole-cell averages. On the other hand, existing cell fractionation techniques allow changes in the very metabolite pools to be measured. A further constraint is that, in addition to the free species (kinetically and thermodynamically relevant), certain metabolites are partly present in protein-bound form. We have addressed compartmentation and protein-binding of NAD(H), a key redox carrier and signaling molecule, by determining the cytosolic free NAD/NADH ratio from the product/substrate ratio of a suitable near-equilibrium redox reaction, which we introduced in yeast specifically for this purpose(1). Using this method we found that the cytosolic free NAD/NADH ratio was more than 10-fold higher than the whole-cell total NAD/NADH ratio. We also demonstrate the use of this method under highly dynamic conditions and discuss the implications of the newly available data for quantitative physiology, particularly in the study of redox metabolism, and thermodynamic reaction network analysis. Another aspect that can be problematic in microbial metabolomics is sampling and sample treatment. Quenching in 60% methanol at -40ºC is the standard method for sub-second arrest of metabolic activity but there have been contradictory reports in the literature on whether metabolites leak from yeast cells. By determining the levels of a large range of metabolites in different sample fractions and establishing mass balances we could trace their fate during the quenching procedure and confirm that leakage does occur, to such an extent that the levels of most metabolites have been previously underestimated by at least 2-fold(2). Using this quantitative approach we evaluated the effect of different quenching conditions and developed a method where leakage is entirely prevented. Making use of improved data on intracellular metabolite levels we re-evaluate the need of sub-second quenching and of removing the extracellular medium. We also discuss the implications of these findings for in vivo kinetic modeling and non-stationary 13C flux analysis.

Hui-Ming Lin, U Auckland, NZ

'Metabolite profiling of a Crohn's disease mouse model for inflammatory biomarkers'

Crohn's disease is a chronic recurring inflammatory disorder of the bowel. The exact disease etiology is unknown, but genetic studies and animal models indicate that the disorder arises from an abnormal immune response towards gut microflora. The IL10 gene knockout mouse (IL10-/- mouse) is one of many Crohn's disease mouse models used to investigate disease pathology and evaluate experimental treatments. Our research aims to identify inflammatory metabolite biomarkers in the IL10-/- mouse by non-targeted metabolite profiling of urine. These metabolite biomarkers will not only improve our understanding of Crohn's disease pathology in the IL10-/- mouse, but will also enable real-time monitoring of disease progression and treatment efficacy. We performed non-targeted gas-chromatography mass spectrometry analysis on urine samples collected from wild-type and IL10-/- mice of C57BL/6 background strain at ages 6, 7.5, 9 and 10.5 weeks old. XCMS software was used to extract and align mass ions from chromatogram data. Multiple T-testing of mass ions revealed 8 metabolites that are consistently different between IL10-/- and wild-type at each sampling event. The identities of these metabolites were confirmed by comparison with authentic standards. These metabolites indicate perturbations in metabolic pathways such as tryptophan catabolism, fatty acid oxidation and protein glycation, which all can be linked to immune function. Work is underway to assess the relation of these metabolite levels to other inflammatory indices and their biological significance in the immune response of the IL10-/- mouse.

Denise Sonntag, Biocrates, Austria

'High altitude effects on the metabolism of healthy mountaineers – a quantitative metabolomics approach to medicine'

Altitude sickness is a phenomenon that can become life threatening, if pulmonary or cerebral edemas develop. The biochemical changes that lead to head ache, dizziness, nausea, etc., and eventually to edema are only partially known. To better understand this multifactorial phenomenon, quantitative targeted metabolomic analysis was used to monitor metabolic changes in sera of healthy mountaineers that occurred during exposition to an altered environment. Metabolic data that reflect dramatic changes in energy metabolism, in the oxidation and content of fatty acids, and in markers of oxidative stress and inflammation in response to high altitude conditions will be presented.

Herbert Hill, Washington State U

'Metabolic Profiling of Human Blood by Ion Mobility Mass Spectrometry'

With increasing necessity to decipher complex biological systems, metabolomics has emerged as a promising tool that compliments those of genomics, proteomics and transcriptomics. However, unavailability of an efficient

instrumental analytical technique that can rapidly analyze complex biological samples containing metabolites of diverse chemical and physical properties hamper rapid characterization of a metabolomes.

This investigation for the first time reports the rapid analysis of human blood metabolome by electrospray ionization ambient pressure ion mobility/time of flight-mass spectrometry (ESI-IMMS) as the analytical method. The time of flight mass spectrometer and supporting electronics were designed and assembled at Ionwerks Inc., Houston. The ESI source, atmospheric pressure IM system, and supporting electronics were designed and assembled at Washington State University, Pullman. The IM system and the Tof MS System were interfaced at WSU.

Human blood metabolites were extracted in hot methanol and subjected to analysis by IMMS. The two dimensional information thus obtained was then used to identify the metabolites present in the sample. The results demonstrate that application of ESI-IMMS to metabolomics not only allows detection of large number of metabolic features but also provides separation of isomeric metabolites in a matter of minutes. Detection of more than 1100 metabolites and separation of approximately 300 isomeric metabolic features in human blood was achieved in 30 minutes by the technique. Estimated concentration of the metabolites range between low micromolar to low nanomolar concentrations. Various classes of metabolites (amino acids, organic acids, fatty acids, carbohydrates, purines and pyrimidines etc) were found to form a characteristic mobility-mass correlation (MMC) curve that aided in metabolite identification. Peaks corresponding to various sterol derivatives, estrogen derivatives, phosphocholines, prostaglandins, cholesterol derivatives detected in the blood extract were found to occupy characteristic two dimensional IMMS space. Low abundance metabolite peaks that can be lost in MS random noise were resolved from noise peaks by differentiation in mobility space. In addition, the peak capacity of MS was increased six fold by coupling IMS prior to MS analysis.

The results demonstrate that ESI-IMMS is a novel and effective two-dimensional technique that can be applied as a powerful analytical tool for rapid and comprehensive profiling of complex metabolomes.

Yoshiaki Ohashi, Human Metabolome Technologies, JP

'Depicting Large-Scale Metabolome Map of Histidine-Starved Escherichia coli by CE-TOFMS'

Metabolic changes in response to histidine starvation were observed in histidine-auxotrophic *Escherichia coli* using a capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS). Prior to the analysis, we prepared an *E. coli* metabolome list of 727 metabolites reported in the literature. An improved method for metabolite extraction was developed, which resulted in higher extraction efficiency in phosphate-rich metabolites. Based on the results, 375 charged, hydrophilic intermediates in primary metabolisms were analyzed simultaneously, providing quantitative data of 198 metabolites. We confirmed that disciplined responses in the glycolysis, the TCA cycle, and amino acid and nucleotide biosynthesis pathways as regulated by amino acid starvation.

David Watson, U Strathclyde, UK

'Applications of hydrophilic interaction fourier transform mass spectrometry in metabolomics'

The output from the Orbitrap FT-MS produces vast data sets and contains thousands of ions, the mass accuracy on the freshly tuned instrument with lock masses applied is consistently < 1 ppm. The clearest success in data processing has been achieved by using Excel based macros written in house. A wide range of model systems and some clinical samples have been examined. The purest demonstration of metabolomics has been in the analysis of metabolic profiles from *Drosophila* mutants where explicable but unexpected changes were observed in metabolic pathways remote to from the metabolic pathway affected by the mutation

Nelly Aranibar, Bristol-Myers Squibb

'Modulation of ascorbic acid metabolism by cytochrome P450 induction revealed by a metabonomics and transcriptomics Approach'

Induction of drug metabolism enzymes has been known for several decades and has vast clinical and pharmacological significance. P450 induction can influence drug-drug interactions, as well as pharmacodynamics and toxic responses to drugs. Enzyme induction usually results from increased gene transcription and de novo protein synthesis, triggered by activation of nuclear receptors. However, it is difficult to predict which particular P450s will be induced from the structure of a xenobiotic, due to co-regulation and "cross-talk" between transcriptional regulatory elements. Presently, enzyme induction is characterized almost exclusively by invasive methods, including quantitation of mRNA levels and by evaluating microsomal activity toward specific substrates.

In this paper, we describe how common agonists of the constitutive androstane receptor (CAR), including phenobarbital (PB), diallylsulfide (DAS) and the CRFR1 (corticotropin releasing factor receptor-1) antagonist DMP-904 alter the urinary excretion of metabolites in the biosysnthesis of ascorbic acid. The effects exhibited dose- and time-dependent patterns, and were correlated to transcriptional changes in enzymes regulating ascorbic acid biosynthesis and reutilization. Finally, beta-naphtoflavone (BNF), an agent that induces CYP1A enzymes via activation of the aryl hydrocarbon receptor (AhR), did not show similar effects, suggesting that changes in ascorbic acid metabolism are not correlated with all metabolizing enzyme inducers. The results illustrate how metabonomic data can be integrated with transcriptional profiles to define the phenotypic changes associated with molecular activation of transcriptional pathways

Prasad Phapale, Kyungpook National University, South Korea
'Potential of pharmacometabolomic approach to study drug response'

The need of individualized drug therapy to get maximum desired therapeutic effects and minimum adverse drug effects is well highlighted in pharmacogenomics. However, the success of the pharmacogenomics also clearly indicated the need of addressing the other environmental factors like metabolomic phenotypes to get enhanced and global understanding of drug response to overcome the current limitations of pharmacogenomics. In pharmacometabolomic approach metabolite profile data along with pharmacogenomic information can be used to predict inter-individual variations in drug response/effect. Thus, combination of pharmacogenomics and pharmacometabolics can significantly contribute to personalized drug therapy.

In this study, we have administered and monitored dose(0.075mg/kg) of Tacrolimus®(an immunosuppressant) in 30 healthy Human subjects. Selection of subjects is done according to CYP3A5 genotypes. All subjects are kept under controlled conditions for 24hrs before dosing to collect pre-dose urine and plasma samples. We analyzed all pre- and post-dose urine and plasma samples by comprehensive liquid chromatography mass spectroscopic (LC-MS) based non-targeted metabolomic profiling methods. In non-targeted metabolomic profiling two complementary techniques RP-LC-MS using C18 column and HILIC-UPLC-MS using HILIC column were used to cover both polar and non-polar metabolites. We have also monitored concentrations of Tacrolimus® in blood samples (collected at different time points) using UPLC-MS/MS in MRM mode and ascomycin as an internal standard to get pharmacokinetic (PK) data. Pre-dose metabolomic data then subjected to data analysis by SIMCA P+ software to generate PCA and PLS score plots which are used for visualization and grouping of this data. These groups are then correlated with other two groups generated from Pharmacokinetic data and from CYP3A5 genotype information. We found overlapping relation among these metabolomic (pre-dose), pharmacokinetic and genotypic groups. Efforts are also made to generate statistical model to improve predictive power of pre-dose metabolomic data to study inter individual variations in drug response.
In conclusion, pre-dose metabolite profile of an individual along with genotype information can predict inter-individual variations in drug response and combination of pharmacometabolomics approach with pharmacogenomics can significantly contribute to the personalized drug therapy

Thomas O'Connell, U North Carolina
'The application of pharmaco-metabonomics to acetaminophen induced hepatotoxicity in humans'

In a recent study by Watkins (JAMA,296,87,2006) it was found that >30% of adults receiving 4gm/day of acetaminophen experienced moderate elevations in ALT levels, indicative of mild liver injury. We investigated the use of pharmaco-metabonomics to distinguish the responders (elevated ALT) from non-responders (no elevation). The urinary metabolome was profiled pre-dose, immediately after dosing and at the end of a 7 day dosing period. Although the pre-dose model was weak, the early dosing model was predictive of responder status well before the ALT elevations occurred. This "early-intervention" pharmaco-metabonomics may be of value in the study of drug toxicity in humans.

Margriet Hendriks, UMC Utrecht, NL
'Metabolic network discovery through reverse-engineering of metabolome data'

Reverse engineering of omics data to infer underlying biological networks is one of the challenges is systems biology. However, applications in metabolomics are rather limited. We focused on a systematic analysis of metabolic network inference from *in silico* data. Different data sets based on biological/environmental variability were analyzed using similarity based network inference approaches. Comparison of inference power of different similarity scores indicated clear superiority of conditioning or pruning based scores. A mathematical measure based on optimal experimental design gives clues in information quality of data sets, while weak interaction strength between connected metabolites can explain why these edges could not be detected.

Katrin Strassburg, MPI-MP, Germany

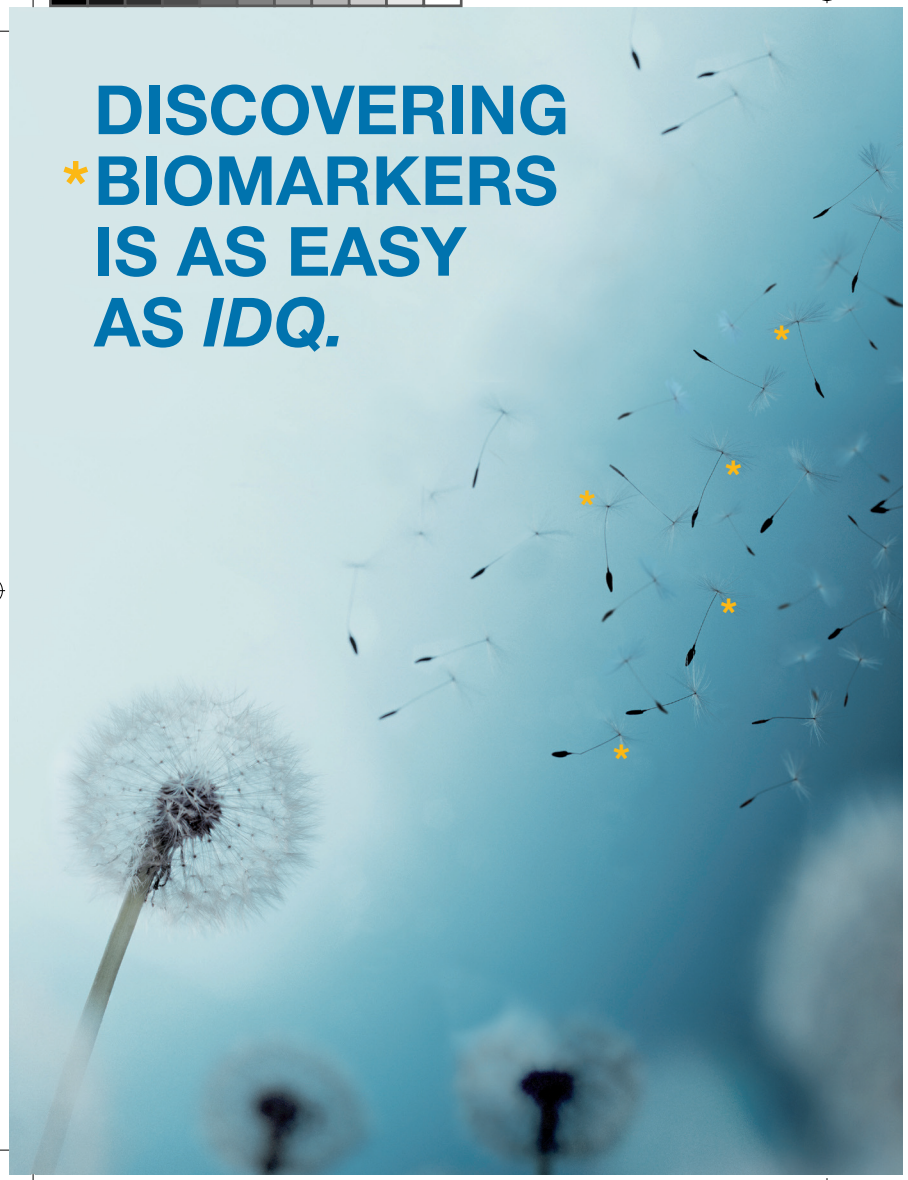
'The temperature stress response of Saccharomyces cerevisiae revisited: Integrative transcriptome and metabolome analysis based on activated metabolic networks'

We present an integrative study of transcript and metabolite pools of yeast responding to high and low temperature. With precise empirical technologies in place local and global properties of biological networks were investigated. We introduce the concept of active networks for the analysis of adaptive dynamic processes projected onto static scaffold networks such as YeastCyc. We discovered global differential network properties which change in response to the applied opposing temperature cues and may be associated with changes in growth rate. Ultimately bridging global and local investigations the time shifted interaction between metabolome and transcriptome will be discussed.

J Scott Breunig, Princeton

'Genetic basis of metabolome variation in yeast'

Our study focuses on inter-individual differences in core metabolism. In humans, these differences underlie susceptibility to diabetes and heart disease. In yeast, they contribute to wine flavor and the efficiency of bioethanol generation. Metabolic quantitative trait loci (mQTL) were found for half of the studied compounds, most having high heritability values. A handful of loci link multiple metabolites. These "hotspots" for genetic control of the metabolome differed from transcriptome and proteome hotspots, suggesting DNA-level changes regulate metabolism specifically, rather than globally altering cellular composition. SNP, conservation, and function data from hotspot genes provide testable hypotheses for understanding genetic regulation.



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Poster Abstracts

Posters:

Presenting authors are asked to be in attendance at their posters at the following times:

Session 1, Thursday 1:00 - 2:30 p.m.

Session 2, Thursday 4:30 - 5:30 p.m.

Session 3, Friday 4:00 - 5:30 p.m.

P001 Session 1, Thursday 1:00 - 2:30 p.m.

Hypoxia and normoxia metabolomics profiling on MDA 231 Breast Cancer Cells

Ching-Hua Kuo (3, 4), I-Ling Tsai (3, 4), San-Yuan Wang (2,4), Y. Jane Tseng (1,2,3,4)

(1) Graduate Inst. of Biomed. Electronic and Bioinformatics, National Taiwan University, Taiwan. (2) Dept. of Computer Science and Information Engineering, National Taiwan University, Taiwan. (3) Department of Pharmacy , National Taiwan University, Taiwan. (4) Metabolomics Group, National Taiwan University, Taiwan

Tumor hypoxia results from insufficient supply of oxygen that compromises biological functions. Tumor hypoxia is considered associated with tumor propagation, malignant progression, and resistance to therapy and it is becoming an important field of research in tumor physiology and cancer treatment. Many studies on hypoxia were performed on the RNA and protein level. We performed metabolomics profiling on MDA 231 Breast Cancer Cells under hypoxia and normoxia condition to further understand the mechanism of tumor hypoxia.

Total of eighteen samples were collected from subculture plates for cell counts of 8×10^5 after two days culture. Each three samples for hypoxia and normoxia were collected at fourth, twenty-fourth, and forty-eighth hour. Cells extracts were measured by both UPLC-MS and 1H-NMR. A multivariate statistical procedure including data normalization on 1H NMR spectra was developed to identify peak positions associated with hypoxia. The metabolomic profiles trend of normoxia and hypoxia after four, twenty-fourth, and forty-eight hours displayed significant different patterns on normoxia and hypoxia cells. Normoxia cells displayed same patterns after twenty-four and forty-eight hours of growth. Hypoxia cells displayed similar pattern of metabolite profiles at four hours and deviated from normoxia cells after twenty-four and forty-eight hours. Marked changes in the levels of primary metabolites, including pyruvate, glucose, alanine, valine, and glutamine were identified. Hypoxia metabolomic profiling reveals glycolysis/glucogenesis pathway dysregulation in breast cancer.

P002 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolic profiling of prostate adenocarcinoma in pre-biopsy urine specimens by ^{1}H-NMR, GC-MS, and LC-MS/MS analysis

Akansh Murthy (1), Theckelnaycke M. Rajendiran (1), Laila M Poisson (1), Javed Siddiqui (1), Robert J. Lonigro (1)

(1) University of Michigan

Prostate Cancer is the second major cause of cancer related death in the US. Prostate Specific Antigen (PSA) levels in the serum are used as the clinical standard for early detection of the disease. However, due to lack of specificity of PSA for prostate cancer, needle biopsy is invariably used to verify the presence of the tumor. This has resulted in a rise in the number of unnecessary biopsies performed which in addition to being invasive are also associated with additional clinical complications. Thus there is an urgent need to develop markers that can supplement PSA in early detection of prostate cancer. Looking at this from the patient's perspective such a test needs to be non-invasive while being able to be adapted in the clinical setting. We begin this biomarker search by interrogating the post-DRE (digital rectal exam) urine metabolome of 14 men who presented elevated PSA levels warranting a prostate biopsy (median PSA: 6.5 ng/mL, range: [4.1, 31.9]). Both HPLC coupled ^{1}H-NMR and mass spectrometry (GC-MS and LC-MS\MS) were used to quantify the metabolic profiles in the urine. Over 75 metabolites have been identified from the ^{1}H-NMR spectra.

Jointly the MS analyses discovered 557 biomolecules, of which 216 were identified, in 12 of the 14 samples. Data from all three methodologies were subjected to compound database/library matching and chemometric analysis.The three technologies provide coincident and complementary biomolecule measurements resulting in a thorough view of the urine metabolome. The samples were selected such that 50% received a positive biopsy result with biopsy Gleason grades ranging from 6 to 9 among the positive cases. We perform both supervised and un-supervised analysis to determine metabolite alterations that could be used to better predict biopsy outcome. Further we define candidate metabolites whose levels are altered in organ confined disease and delineate pathways that are altered during prostate cancer development.

P003 Session 3, Friday 4:00 - 5:30 p.m.

Spatial metabolic fingerprinting by Raman chemical mapping: application to human cervical cancer cell lines exposed to Indinavir and Lopinavir

Dong-Hyun Kim (1), Roger Jarvis (1), Lynne Hampson (1), Ian N Hampson (1), Anthony Oliver (10), Royston Goodacre (1)

(1) University of Manchester

Cervical cancer is the second most common form of cancer diagnosed in the UK, affecting many hundreds of women with an annual mortality rate of approximately 1000 individuals. It is known that the human papilloma virus (HPV) can cause cervical cancer, where the pathogenesis of high-risk HPV arises from expression of E6 oncoproteins. This results in the induction of improper activity of the 26S proteasome which leads to the degradation of the tumour suppressor p53 and other cellular proteins. Recently, it has been reported that the drugs Indinavir and Lopinavir, which are currently used as a human immunodeficiency virus (HIV) protease inhibitor, could also inhibit E6-mediated proteasomal degradation of mutant p53 in E6-transfected C33A cells.

Raman micro-spectroscopic imaging is a powerful and non-destructive tool based on molecular vibrations induced by the illumination of the sample with laser light (usually in the visible to near IR) which results in inelastic light scattering. This Raman scattered light gives molecular information about the sample and can be used spatially to resolve changes in the chemical composition of a cell, as well as the distribution of drugs inside cells.

C33A vector control and E6-transfected cells were seeded in the complete medium onto CaF2 discs overnight at 37°C, 5% CO2. After this period, cells were exposed to 1mM of Indinavir and 30M of Lopinavir for 24 h at 37°C. Cells were fixed in ice cold methanol then washed using PBS. The cells were then analysed using Raman micro-spectroscopy with an excitation wavelength of 830 nm and laser power at the sampling point of ~5mW. Chemical image maps were generated for a variety of univariate metrics including peak area and S/N ratios, in an attempt to understand the complex metabolic changes occurring in these cells, and the chemical distribution and localisation of the drugs.

P004 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolic footprinting using FT-IR spectroscopy on human cervical cancer cell lines exposed to Indinavir and Lopinavir.

Dong-Hyun Kim (1), Roger Jarvis (1) Lynne Hampson (1), Ian N Hampson (1), Royston Goodacre (1)

(1) University of Manchester

Cervical cancer is a major gynaecological cancer among women diagnosed in the UK. Each year in the UK over 2,700 women are diagnosed and approximately 1,000 deaths are caused by this disease. The global figures are even more astounding, with an estimated 493,000 women affected by cervical cancer and 273,500 deaths each year.

It is known that the human papilloma virus (HPV) can cause cervical cancer, where the pathogenesis of high-risk HPV arises from expression of E6 oncoproteins which induce improper activity of the 26S proteasome thus leading to the degradation of the tumour suppressor p53 and other cellular proteins. Recently, it has been reported that the drugs Indinavir and Lopinavir, which are currently used as a human immunodeficiency virus (HIV) protease inhibitor, could also inhibit E6-mediated proteasomal degradation of mutant p53 in E6-transfected C33A cells.

FT-IR spectroscopy is a powerful tool for the global, sensitive and highly reproducible physico-chemical analysis of biological systems and is very rapid due to the automated high-throughput processing of biological samples (typically 10s - 1min per sample arranged on 96 well plates). In addition this reagentless method requires minimal sample preparation and no chemical derivatisation like GC-MS. Therefore, we believe this technique is an effective non-invasive method for the analysis of extracellular metabolites secreted from cells into their growth medium and we plan to exploit this in order to understand biological consequences of cellular exposure to anti-viral drugs.

C33A parent, vector and E6-transfected cells were seeded in twelve 6-well culture dishes to give three biological replicates for each treatment and allowed to adhere overnight at 37°C, 5% CO2. Indinavir (0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7 0.8, 0.9 and 1mM), Lopinavir (0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30M), or control water and DMSO were added to the relevant wells and cells were incubated for 24h at 37°C, 5% CO2. 1ml of the growth media were then collected and centrifuged at 3000g for 10min at 4oC to remove any cells and cell debris. Supernatants were collected and 40 ul of each were pipetted on a 96 well zinc selenide (ZnSe) plate. All FT-IR spectra were obtained in the 4000 - 600 cm-1 range using 64 scans at 4 cm-1 resolution. Spectra acquisition and spectral corrections were carried out using OPUS software 5.5 and then imported into Matlab 6.1 software for further spectral analysis. Canonical variates analysis (CVA) was used to group the samples on the basis of the retained principal components (PCs) and the priori knowledge of which spectra were biological triplicates. In addition, partial least squares (PLS) regression analysis was performed for predictive linear modelling to investigate the relationship between the spectral variations of the culture media.

P005 Session 2, Thursday 4:30 - 5:30 p.m.

NMR and GC/MS Urinary Metabolic Profiling of Bladder Carcinoma

Kishore Kumar Pasikanti (1), Eng Shi Ong (1), Kesavan Esuvaranathan (2), Revathi Kamaraj (2), Eric Chun Yong Chan (1)

(1) National University of Singapore (2) National University Hospital Singapore

Background: Bladder cancer is one of the more common cancers diagnosed in clinics with an estimated 68,810 new cases in 2008 in the United States. Bladder cancer is characterized by frequent recurrences that can progress to more invasive disease. The recurrence rates for bladder cancer are as high as 50-70%, therefore, life-long close surveillance is needed which renders bladder cancer a very expensive cancer to treat. The surveillance generally requires cystoscopy to be

performed frequently. Although many other urinary assays have been utilized, most of the diagnostic assays do not achieve the specificity and sensitivity of cystoscopy. Therefore, alternative non-invasive diagnostic methods such as the use of biomarkers become important. We hypothesize that the human urinary metabolic profiles may be used to identify bladder cancer patients from healthy controls. In this pilot study, nuclear magnetic resonance spectroscopy (NMR) and gas chromatography/mass spectrometry (GC/MS) were evaluated for the detection of bladder cancer using the metabonomic approach.

Methods: Urine samples were collected from 10 bladder cancer patients (BC) and 10 cancer-free controls (H). All the urine samples were analyzed by 500 MHz NMR and GC/MS after suitable pretreatments. The respective data were analyzed using principal component analysis (PCA) followed by orthogonal partial least squares analysis (OPLS). All variables were mean-centred and unit variance scaled. Additionally, orthogonal signal correction (OSC) was used to optimize group separation. About 70% of the samples (the 'training set') were selected to construct an OPLS model that was used to predict the class membership of the remaining 30% of samples (the 'test set'). Various combinations of training and test sets were investigated to evaluate the predictive ability of the resulting OPLS model.

Results: The OPLS model calculated from OSC-filtered NMR data for the training set accurately predicted the classification of the test samples as BC or H. The sensitivity and selectivity of the OPLS model were 100% for different combinations of samples for building training sets and test sets. The OPLS model generated using the GC/MS data was also found to be complementary to the NMR technique.

Conclusions: Our findings clearly demonstrate the potential of urine metabonomics to discriminate bladder cancer patients from healthy individuals. The good prediction abilities of the OPLS models generated by NMR and GC/MS analysis serve as strong evidence that urinary metabolic profiling can aid in the biomarker research of bladder cancer and potential development of a clinically applicable noninvasive diagnostic assay.

P006 Session 3, Friday 4:00 - 5:30 p.m.

Extra cellular metabolomics of non tumorigenic and tumorigenic human uroepithelial cells

Kishore Kumar Pasikanti (1), Juwita Norasmara (2) , a Mahendran (2) , Kesavan Esuvaranathan (2) , Eric Chun Yong Chan (1)

(1) National University of Singapore (2) National University Hospital

Bladder cancer is one of the more common cancers diagnosed in clinics with an estimated 68,810 new cases in 2008 in the United States. Although many urinary assays have been utilized, most of the diagnostic assays do not achieve the specificity and sensitivity of cystoscopy. Therefore, alternative non-invasive diagnostic methods such as the use of biomarkers become important. While *in vivo* clinical samples are valuable to determine directly end-point biomarkers related to bladder cancer, *in vitro* cell culture samples might be important to identify biomarkers related to the proliferation of native cells. *In vitro* biomarkers arising directly from the cancer cells may facilitate the mechanistic understanding of the occurrence of biomarkers clinically. In this study, we show the application of GC/MS-based metabolomics to investigate the metabolic profiles of non-tumorigenic human uroepithelial cells (HUCT1) and tumorigenic human uroepithelial cells (HUCT2) cultures. We have used a metabolic profiling approach to test the hypothesis that metabonomic signatures of primary metabolites can be used to characterize the molecular changes.

Methods: Both HUCT1 and HUCT2 cell lines were cultivated in 1 mL of Ham's F-12 media supplemented with 10% fetal bovine serum (FBS) at 37oC with 5% CO2. 24 hrs after the cells were seeded, the media was removed and replaced with fresh Ham's F-12 media and incubated for further 24 hrs. The cell culture supernatants were then harvested in pre-chilled tubes on ice. A 100 µL of cell culture supernatant was protein precipitated using methanol and an aliquot of the mixture was then separated, dried, trimethylsilyl (TMS) derivatized and 1.0 µL of the derivatized extract wasinjected into the GC/MS system via split injection (1:5). Metabolic profiling of HUCT1 and HUCT2 samples in combination with multivariate data analysis techniques was performed to evaluate the changes in metabolome. HUCT1 and HUCT2 cells were cultured in six replicates on two different occasions to assess the intra and inter-day reproducibility of the results.

Results: Principal component analysis (PCA) showed clear separation of two groups. Subsequent partial least-squares-discriminant analysis (PLS-DA) revealed differences between metabolite profiles of HUCT1 and HUCT2 cell cultures. Several metabolites were identified which were statistically significant in two groups. This model contained two components, showing the performance statistics of R2X = 0.602, R2Y = 0.964 and Q2 (cumulative) = 0.826. In addition, clustergram was plotted to perform hierarchical clustering analysis on HUCT1 and HUCT2 samples. Clear clustering of HUCT1 and HUCT2 samples was also observed in clustergram. Rank correlation matrix was built to access the pair-wise linear correlation between GC/MS analysis of all the HUCT1 and HUCT2 replicates. The correlation map revealed that the replicates of HUCT1 and HUCT2 are highly correlated (pairwise correlation coefficient >0.97) within each group.

Conclusions: Results from the present study clearly demonstrate that it is possible to distinguish the metabolic profiles of tumorigenic and non tumorigenic uroepithelial cells using GC/MS analysis of culture media and chemometric data analysis.

P007 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolomics and Chemometric Bionformatics to find Markers for Tumor Detection, Prognosis, Progression and Theraphy Prediction: prostate, ovarian and brain Malignancies

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(1) Umeå University (2) Swedish University of Agricultural Sciences

Better markers within the fields of prostate, ovarian, and brain malignancies for early tumor detection, prognostication, progression, and therapy prediction are urgently needed. Ideal tumor markers should be tumor specific and assessable in blood. Today clinically available tests for these malignancies are very unspecific.We are performing Metabolomic (GC-TOFMS and UPLC-MS) based studies of tumor tissue and plasma from case-control series and experimental models. This is combined with the application of bioinformatics tools based on chemometric methodology. In parallel, to this proteomic-(2-D DIGE, SELDI-TOF, tissue-array), and genomic- (expression array) based are being carried out and will be evaluated together with the metabolomics data. Our hypothesis is that we will find biomarkers in the tissue, which are detectable in blood, and related to cancer-specific survival, recurrence-free time after therapy, or tumor characteristics of prognostic value. Models are built from training data sets and candidate markers will be identified and validated in separate test sets including prospective samples. We have available exclusive biobanks of tumor tissue as well as single and repeated prospective blood samples from cancer cases in the population-based Northern Sweden Health and Disease Study. For a substantial number of these patients we have also collected fasting blood samples before surgery, diagnostic tumor biopsies, tumour and normal tissue at surgery, as well as blood samples after surgery. This gives unique

opportunities to study metabolic, protein and gene expression patterns in pre-diagnostic blood samples, how these patterns evolve over time, and how they relate to patterns in tumor tissue. So far, preliminary results have revealed that metabolomic patterns in plasma seem to be related to progression of prostate cancer (Pca). Using Chemometric Bioinformatics we have been able to discriminate between healthy subjects and Pca cases by using extracted information from GC-MS based studies. In addition, even more interestingly, GC-MS metabolite data obtained before radical prostatectomy seems to be predictable for prostate specific antigen (PSA) relapse after surgery.

P008 Session 2, Thursday 4:30 - 5:30 p.m.

Effects of exercise on tumor development in APCmin mouse model of colon cancer. Metabolomics study.

Ilya Bederman

Apologies for the missing abstract, the file was corrupted in the database

P009 Session 3, Friday 4:00 - 5:30 p.m.

Targeted profiling and analysis of urine metabolome to identify potential biomarkers associated with cancer cachexia

Jianguo Xia (1), David Hau (1), Roman Eisner (1), Cynthia Stretch (1), Vickie Baracos (1)

(1) University of Alberta

Cancer cachexia is a complex metabolic syndrome characterized by significant weight loss and muscle depletion. Despite decades of clinical investigations, the mechanisms leading to cancer cachexia remain unclear and multifactorial. Recent advances in high-throughput metabolomics have provided opportunities to comprehensively investigate metabolic alterations in this syndrome.

In this paper, we describe a systematic analysis of urine metabolomics data from 57 cancer patients with different degrees of muscle mass changes. 1H nuclear magnetic resonance (NMR) spectra were collected and processed. Fifty-four common metabolites were identified and quantified via targeted profiling techniques. Three different approaches, including a robust statistical method using significance analysis of microarray (SAM), a machine learning algorithm based on random forests (RF), and the conventional chemometrics method using partial least squares (PLS), were explored to perform feature selection and ranking. A panel of 10 metabolites was identified to be significantly associated with cancer cachexia. Most of them are involved in energy or amino acid metabolic pathways. Using the selected metabolites, a good separation pattern was obtained in principal component analysis (PCA), and a naïve Bayesian (NB) classifier could achieve 92.98% predictive accuracy based on leave-one-out cross validation.

Targeted urine metabolomics is a useful technique for investigating metabolic alterations in cancer cachexia. The significant overlap among the metabolites identified by three different approaches suggests an increased reliability over the association between these compounds and the disease. These metabolites are linked to the known biochemical pathways consistent with current theories on the etiology of cancer cachexia. Some of them may serve as potential biomarkers to aid in early diagnosis of this disease. The statistical and machine learning methods assessed in this paper can be used as complementary and alternative methodologies to support current strategies for metabolomics data analysis.

P010 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolomic Profiling of Prostate Cancer Progression

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(1) University of Michigan

Multiple, complex molecular events characterize cancer development and progression. Deciphering the molecular networks that distinguish organ-confined disease from metastatic disease may lead to the identification of biomarkers of cancer invasion and disease aggressiveness. Although gene and protein expression have been extensively profiled in human tumors, little is known about the metabolomic alterations that characterize neoplastic progression. Using a combination of high throughput liquid and gas chromatography-based mass spectrometry, we measured 626 metabolites across 42 tissue samples related to prostate cancer progression. Prostate cancer progression was characterized by alterations in amino acid metabolism and methylation potential. The increase in methylatation potential is reflected in elevated levels of methylated metabolites that have been characterized for their role in prostate cancer progression. Importantly, these metabolites were also elevated in urine from biopsy-proven prostate cancer patients making it a potential candidate for non-invasive detection of the disease. Results pertaining to functional characterization of these metabolites in context of prostate cancer progression and validation of their biomarker potential are discussed.

P011 Session 2, Thursday 4:30 - 5:30 p.m.

LC-ESI-MS/MS method for monitoring of oxidative stress markers

Marek Kuzma (1), Kamila Syslova (2), Petr Kacer (2), Jindriska Lebedova (3), Zdenka Fenclova (3)

(1) Institute of Microbiology (2) Institute of Chemical Technology Prague (3) First Faculcy of Medicine

Oxidative stress results from an imbalance between production of free radicals and antioxidant defenses. With the cells' natural antioxidant mechanisms overwhelmed, the excess reactive oxygen species may cause radical-mediated damage to biomolecules. In humans, oxidative stress is involved in many diseases, such as atherosclerosis, Parkinson's disease and Alzheimer's disease, but it may also be important in prevention of aging.

The analytical procedure for monitoring of 8-isoprostane, 4-hydroxynonenal and malondialdehyde as a group of oxidative stress markers in various body-fluids (blood plasma, urine, exhaled breath condensate) was developed. The method combines separation method and/or derivatization procedure with LC-ESI-MS/MS. It was tested several reactions for derivatization of aldehydes. The developed method was used for rapid and highly effective separation and quantification of the above-mentioned biomarkers present in low concentrations in such biological matrixes. Pretreatment method comparison is presented as well as the solution for quantification of rapidly degradated markers after sampling from the human body. LC-ESI-MS/MS operated in multiple reactions monitoring (MRM) mode was used for its exceptionally high degree of selectivity, and stable-isotope-dilution assay for its high precision of quantification. The combination of mass spectrometry detection with separation techniques (HPLC) enabled retention the analytes from the solvent front and avoids co-elution of salts and endogenous matrix components which can suppress

the ionization of the analyte during the ESI. The developed method allowed unequivocal parallel determination of oxidative-stress biomarkers at the same run. The method was optimized and validated. The imprecision of the method ranged between 6 – 14 %. Finally, the method was tested on real samples collected from patients with different oxidative stress induced diseases (silicosis, asbestosis) and on the control group of healthy subjects.

Acknowledgement: The authors thank the Ministry of Health of the Czech Republic (Grant NR-9338-3/2007) for financial support.

P012 Session 3, Friday 4:00 - 5:30 p.m.

Early identification of Familial Dilated Cardiomyopathy by integrating MRI Imaging and LC/MS lipidomics data

Marko Sysi-Aho (1), Juha Koikkalainen (1), Tuulikki Seppänen-Laakso (1), Jyrki Lötjönen (1), Tiina Heliö (2)

(1) VTT Technical Research Centre of Finland (2) HYKS Hospital District of Helsinki and Uusimaa, Cardiology Department

Dilated Cardiomyopathy (DCM) is an important cause of heart failure and it is characterized by enlargement and impaired contraction of left or both ventricles. So far, the most important risk gene for cardiomyopathy is the lamin A/C gene (LMNA). Recently Koikkalainen et al. [1] compared 12 LMNA mutation carriers that were asymptomatic, but at risk of developing DCM, to a group of 14 matched control subjects using clinically relevant parameters derived from MRI images. They found marked differences in the parameters of the two groups. Our objective is to link lipid data to the heart MRI parameters. Using LC/MS platform we obtained lipid profiles from blood serum samples of the 12 LMNA carriers and a group of 12 controls that participated in the study by Koikkalainen et al. We found marked differences between the cases and matched controls also in their lipidomic profiles. We highlight lipid groups that are associated with certain MRI parameters by visualizing the correlations between lidid data and the MRI parameters. Furthermore, we use multiple regression models to assess the ability of a group of lipids to predict values of selected heart MRI parameters of clinical relevance and vice versa. Our findings may enrich the understanding of the physiology behind LMNA carrying DCM risk subjects and lead to novel strategies for preventing the development of DCM.

[1] J.R. Koikkalainen, M. Antila, J.MP. Lötjönen, T. Heliö, K. Lauerma, S.M. Kivistö, P. Sipola, M.A. Kaartinen, S.TJ. Kärkkäinen, E. Reissell, J. Kuusisto, M. Laakso, M. Oresic, M.S. Nieminen, K.J. Peuhkurinen, Identification of Early Familial Dilated Cardiomyopathy by Determination of a Disease State Parameter Using Cine MR Imaging Data, Radiology, In press.

P012-2 Session 2, Thursday 4:30 - 5:30 p.m.

SDMA as a renal marker

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Patients with chronic renal failure (CRF) are in the highest risk group for the development of cardiovascular disease (CVD). Recent guidelines from American Heart Association (AHA) have

defined CRF as a CVD equivalent. There is a growing body of evidence showing CKD and CVD are associated with deranged nitric oxide (NO) metabolism. While methylated arginine derivative, ADMA (asymmetric dimethylarginine) is shown to be an endogenous inhibitor of NO synthase, its structural isomer SDMA (symmetric dimethylarginine) does not directly interfere with NOS activity. However, SDMA is a potent competitor of L-arginine (substrate for NOS) transport, impairing NO production. It is estimated that humans generate approximately 300 mmol of ADMA per day of which about 85% metabolized by dimethylarginine dimethylaminohydrolase (DDAH). While DDAH I and II hydrolyze ADMA, SDMA seems to be strictly eliminated by renal excretion. The methylated arginines derive from intranuclear methylation of L-arginine residuals by protein-arginine methyltransferase (PRMT) and are released into cytoplasm after proteolysis. While there are several studies establishing ADMA as an effective biomarker for endothelial dysfunction in humans with CRF, the role of SDMA as a marker is still being investigated. In the current study, we have established SDMA as a potential marker for renal function in cats and dogs. A highly sensitive, specific and selective analytical method based on LC /MS has been developed for the quantification of SDMA in serum samples. The LC separation was achieved by using silica column. The API 2000 triple quadrupole mass spectrometer (Applied Biosystems/MDS Sciex) was operated in multiple reaction monitoring (MRM) mode with positive electrospray interface. The MRM transition for SDMA was observed at m/z 203.2 ---> 172.1. An ELISA for SDMA for serum samples is currently under development.

P013 Session 1, Thursday 1:00 - 2:30 p.m.

Training-induced effects on plasma metabolomics in patients with chronic obstructive pulmonary disease

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Rationale: Patients with chronic obstructive pulmonary disease (COPD) show abnormal adaptations of skeletal muscle redox status after training. The phenomenon is clearly more evident in COPD patients with muscle wasting. The analysis of small-molecule metabolite profiles (metabolomics) may shed further light on the adaptations of COPD patients to training. We aimed at analyzing plasma metabolomic changes after 8-week endurance training.

Methods: We studied 13 patients with stable COPD and normal fat free mass (COPD_FFMN 68±4 yrs, FEV1 49±8% pred), 6 COPD patients with low FFM (COPD_FFML 69±11 yrs, FEV1 41±16%) and 12 health sedentary controls (H 65±9 yrs, FEV1 107±14%). Pre- and post-training plasma samples at rest and after constant-work rate exercise at 80% of pre-training VO2peak were analyzed by 1H-NMR spectroscopy. Principal component analysis (PCA) was done. Metabolic effects of training were studied using a kinetic model of central metabolism.

Results: Training-induced enhancement of both VO2peak and 6MWD was seen in all groups ($p<0.01$). At rest, PCA revealed different metabolomic patterns among COPD_FFMN, COPD FFML and H. In all cases, acute exercise (pre- and post-training) generated clear metabolomic changes. While PCA showed similar training-induced metabolic profile between COPD_FFMN and H, COPD_FFML did not present significant metabolomic improvement. Model analysis revealed that training induces more effective oxygen metabolism in healthy individuals than in severe COPD patients.

Conclusions: Metabolomics differentiates the 3 groups at rest and indicates that training improves COPD_FFMN but not COPD_FFML response to exercise. Integration of clinical information with other

"omics" data (functional genomics and proteomics) may help to understand the underlying mechanisms of muscle wasting in COPD and perform early identification of those COPD patients with poor prognosis because of systemic effects.

Supported by grants from BioBridge (FP6-2005- 037909) FIS (2005-061510) and ERS-SEPAR fellowship grant #191

P014 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolite profiling analysis of Methylobacterium extorquens AM1 by a comprehensive metabolomic approach

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(1) University of Washington

Methylobacterium extorquens AM1 is a facultative methylotroph that utilizes the serine cycle for C1 assimilation. In addition to the C1 compounds (methanol or methylamine), M. extorquens AM1 is able to grow on C2 (ethylamine or ethanol) and C4 (succinate) compounds. While the comparison of growth on methanol and succinate has been characterized at a systems level, little is known about global metabolic regulation for growth on ethylamine (C2 compound). To better understand C2 metabolism in M. extorquens AM1, in this study, the methods of comprehensive two-dimensional gas chromatography coupled with time-of-flight mass spectrometry (GCGC-TOFMS) and liquid chromatography-tandem triple quadrupole mass spectrometry (LC-ESI-MS/MS) were developed and optimized in this bacterium. Accurate peak quantitation of intracellular intermediates, including major organic acids and amino acids were determined by GCGC-TOFMS and parallel factor analysis (PARAFAC) software. Central nucleotide and coenzyme A derivatives metabolism were quantified by sensitive and specific multiple reaction monitoring (MRM) using hydrophilic interaction and reverse phase chromatography-MS/MS. When applied to extracts of M. extorquens AM1 grown on ethylamine vs. succinate, some important intermediates were identified to be statistically differentially expressed. Intracellular ATP levels were higher during growth on succinate, while the pools of ADP, AMP and NAD were higher during growth on ethylamine. Cell grown on ethylamine have more than 2-fold higher butyryl-CoA pool and about 1.5 fold higher propionyl-CoA pool compared to cell grown on succinate, while succinyl-CoA, acetyl-CoA and hydroxybutyryl-CoA pools are about 50% lower than cell grown on succinate. Some of the amino acids and organic acids involved in tricarboxylic acid (TCA), serine cycle and ethylmalonyl-CoA pathway are not significantly different between succinate growth and ethylamine growth, while some key intermediates including fumaric acid, malic acid, methylsuccinic acid, phosphoenolpyruvic acid and serine, etc. are obviously elevated or reduced in ethylamine cultures. These differently expressed compounds have provided clues to evaluate key similarities and differences in metabolic pathway between grown on C2 compound and C4 compound.

P015 Session 3, Friday 4:00 - 5:30 p.m.

Metabolomics study of CHO cell cultures

William P.K. Chong (1), Poh Choo Toh (1), Niki S.C. Wong (1), Miranda G.S. Yap (1)

(1) Bioprocessing Technology Institute

Chinese Hamster Ovary (CHO) cells are commonly grown in cultures to express recombinant proteins. Currently, besides lactate and ammonia, there is limited understanding of the metabolites that are released by CHO cells, and their impact on cell growth and recombinant protein production. We present the development of a metabolomics platform to identify other extracellular metabolites in the culture medium.

The platform involved using high performance liquid chromatrography connected to a LTQ-Orbitrap mass spectrometer (MS) for the detection of small molecules at high mass accuracy (<3ppm) and resolution (30,000). Culture medium of CHO cells producing humanized antibodies against the Rhesus D antigen (CHO mAb) from a fed-batch bioreactor culture was collected and analysed. Pair-wise differential analysis was performed to compare fresh medium with day six culture medium, day six being the transition point between exponential and stationary phase of growth. 518 mass peaks were detected that were at least 1.5 fold higher ($p<0.05$) than fresh medium, of which 51 could be assigned a putative identity when matched to the Human Metabolome Database. The metabolite identities were verified as far as possible with isotopic pattern and comparison of MS2 fragments with theoretical fragments or fragments from standards where available. Eight of the 51 mass peaks were amino acid derivatives, some of which are associated with metabolic diseases in humans. An example is glutamylphenylalanine (13,400 fold change) which is elevated in urine of patients who have impaired ability to metabolize phenylalanine. This suggests an excess supply of phenylalanine in the CHO mAb culture, highlighting the need to fine tune the feeding of amino acids to prevent the accumulation of derivatives that can potentially impact cell growth adversely. Another amino acid derivative which could affect cell growth was asymetric-dimethyl-L-arginine (ADMA, 560 fold change). ADMA is a by-product of protein degradation and is known to induce apoptosis in human endothelial cells. The effects of the amino acid derivatives on CHO mAb cell growth and recombinant protein production will be the subject of future studies.

P016 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolite profiling of heterologous proteins expressing Streptomyces lividans TK24 GC-TOF-MS

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(1) University of Manchester

Streptomyces produce a variety of secondary metabolites with commercial applications especially in the pharmaceutical and agricultural industrials. Metabolomics of protein expressing streptomycetes can potentially identify biosynthetic bottlenecks and develop models for the efficient expression and secretion of desired heterologous protein products. We compare the metabolite profiles of Streptomyces lividans TK24 secreting mouse tumour necrosis factor alpha (mTNF-alfa) and xyloglucanase with the wildtype strains. Sixty-eight (68) metabolites showed statistical significant difference between different strains as well as with the length of fermentation The highest levels of several amino acids, sugars and sugar phosphates were detected in protein expressing and secreting strains relative to the non-secreting wildtype. The metabolism of some of these metabolites has

been shown to correlates to the protein biosynthesis as well as generating ATP to drive extracellular secretion in other bacterial species

P017 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolomics study of antibody production in CHO cell cultures

Ying Swan Ho (1), Yih Yean Lee (1), Poh Choo Toh (1), Miranda G.S. Yap (1)

(1) Bioprocessing Technology Institute

The trend of increasing demand for monoclonal antibody (mAb) therapeutic products today highlights the need to constantly improve the efficiency and yield of industrial mAb production from mammalian cells. The design of culture media is one of the key approaches taken to increase the mAb production yield. As such, a better insight into the key metabolic pathways that influence the mAb production process can serve as a good guide for the media design process.

In this study, a metabolomics approach is used to derive a better understanding of the metabolites that are associated with high specific antibody productivity (qAb). Six antibody producing clones with differing qAb were derived from a CHO cell line and adapted to grow in protein-free chemically defined (PFCD) media. Extracellular medium from batch bioreactor cultures of all clones were obtained daily and analysed using a platform which consisted of a high performance liquid chromatography unit connected to a LTQ Orbitrap mass spectrometer (LC-MS). The LC-MS data was pre-processed with SIEVE software (Thermo Corp., US) and a correlation study based on Pearson's coefficient was carried out between the time profiles of the mass peaks and the qAb trend for each clone. 21 mass peaks whose putative identities were assigned by mass comparison with the Human Metabolome Database were found to be positively correlated with qAb in 3 or more of the clones. 11 of the 21 mass peaks comprised of metabolites associated with amino acid metabolism, of which components from the tryptophan and cystine pathways were most significant. Additionally, the study highlighted the possible importance of riboflavin, a media component, in the determination of qAb. The results obtained demonstrate how a "global" metabolomics approach can be a potentially useful guide for the design of optimal media.

P018 Session 3, Friday 4:00 - 5:30 p.m.

Metabolite profiles correlate with drought tolerance characteristics in wheat

Jairus Bowne (1), Juan Juttner (1), Thorsten Schurbusch (1), Tim Erwin (1), Ute Baumann (1)

(1) Australian Centre for Plant Functional Genomics

Metabolomics is used as a functional genomics approach to increase our understanding of how plants are able to adapt to or tolerate many environmental stresses. Drought has a severe impact on agricultural productivity world-wide, and a metabolomics approach may provide an insight to the mechanisms that plants develop to tolerate drought conditions, ultimately leading to the production of new drought tolerant crops that are better adapted to growth in drought conditions. Three wheat cultivars (Excalibur, Kukri and RAC875) that have different drought tolerance characteristics were grown under cyclic water deficiency conditions, and their metabolite profiles were compared to control plants grown under continuously well watered conditions. This detailed investigation of the effects of drought stress on the global metabolic networks allowed the identification of metabolites that were affected by drought stress in all three cultivars, as well as metabolites that changed in a

cultivar-specific manner. The relative tolerance to drought of the cultivars correlated well with the drought-related metabolite changes. Cultivar-specific changes indicated different mechanisms of drought tolerance in two cultivars. In addition, transcriptomic data generated from the same sample material are being correlated with the metabolite profiles which will link metabolites and genes to adaptation and tolerance mechanisms. The genes identified in this manner will be used in future breeding programs to develop crops more tolerant to drought stress.

P019 Session 1, Thursday 1:00 - 2:30 p.m.

The use of environmental metabolomics to determine glyphosate level of exposure in rapeseeds (Brassica napus L)

Giorgio Tomasi (1), Jan H Christensen (1), Iben L Petersen (2), Esther Sørensen Boll (1), Hans C B Hansen (1)

(1) The Faculty of Life Sciences, University of Copenhagen (2) Carlsberg Research Center

In this presentation, plant metabolic fingerprinting is investigated as a tool to detect patterns of plant biomarkers that give a stable and robust response even at low levels of exposure.

As a study case, plant crude extracts from rapeseed seedling (Brassica napusL.) exposed to sublethal concentrations of glyphosate ((N-Phosphonometyl)glycine – also known as Round-up®) were used. Glyphosate affects the shikimic acid pathway, resulting in an accumulation of shikimate, a well-known plant biomarker for exposure to this herbicide. So far, the discovery of new biomarkers for glyphosate exposure focused on selected metabolites downstream from the primary affected metabolite. This approach, however, has a limited capacity for discovering new biomarkers. Conversely, following glyphosate exposure, a great range of other compounds is also affected due to metabolic regulations and therefore it is expected that the metabolic fingerprint would change appreciably and according to the level of exposure.

In this work, chemometric data analysis was used to determine glyphosate level of exposure in rapeseeds and to identify new potential plant biomarkers. All the steps of the analysis were considered: the processing of raw data, the use of ANOVA filtering to perform variable selection and the final multivariate data analysis.

The results demonstrate that metabolic fingerprints combined with multivariate data analysis enable the differentiation between glyphosate exposed plants and controls, as well as the identification of the peaks responsible for it; disclosing great perspectives for the use of environmental metabolomics in risk assessment procedures.

P020 Session 2, Thursday 4:30 - 5:30 p.m.

Functional analysis of rice genes via metabolite profiling by the FOX (Full-length cDNA OvereXpressor) hunting system

Miyako Kusano (1), Doris Albinsky (1), Mieko Higuchi (1), Naomi Hayashi (1) Makoto Kobayashi (1)

(1) RIKEN Plant Science Center

The complete sequencing of the rice (*Oryza sativa* L. ssp. *japonica* cv. Nipponbare) genome by the International Rice Genome Sequencing Project (IRGSP) will lead to powerful functional genomics

approaches. Metabolomics allows comprehensive phenotyping, filling a niche for functional genomics. As a gene specifying method for metabolite phenotyping, we applied the FOX (Full-length cDNA OvereXpressor) hunting system, which is an alternative gain-of-function gene hunting technique. By using this system, rice full-length cDNAs can be over-expressed ectopically into Arabidopsis (Rice-Arabidopsis FOX). For the screening of aerial parts of Rice-Arabidopsis FOX lines, we performed metabolite profiling by using gas chromatography – time-of-flight mass spectrometry (GC-TOF/MS) analysis. We investigated 350 independent Rice-Arabidopsis FOX lines for a change in their metabolite profiles. In these lines, the metabolite changes in 29 lines could be confirmed. It is worth mentioning that 6 lines with a wild type phenotype showed the changed metabolite profiles when compared with empty-vector control lines. In the candidate lines analyzed, we found one overexpressor line which harbors a rice cDNA highly homologous to the Arabidopsis *LBD37* (Lateral Boundaries Domain) gene belonging to a newly characterized family of plant-specific LOB (Lateral Organ Boundaries) protein domain coding genes with widely unknown functions. Analysis of Arabidopsis lines, ectopically expressing the At*LBD37* gene, revealed similar metabolic changes as found in the Rice-Arabidopsis FOX-line. Importantly, both overexpressor lines exhibit the same characteristic hyponastic leave phenotypes. These results suggest that the *LBD37* gene function is conserved throughout both plant species. Moreover, the integrated analysis for metabolite and transcript profiling data of the Rice-Arabidopsis *LBD37* line showed that a part of the observed metabolite changes in this line were likely to derive from changes in transcript levels.

P021 Session 3, Friday 4:00 - 5:30 p.m.

Regional variations in ^{1}H MRS spectra from normal human brain at 3T - a pattern recognition approach

Maria Sokol (1), Agnieszka Polnik (1)

(1) Institute of Oncology

Proton magnetic resonance spectroscopy (^{1}H MRS) in vivo is a non-invasive technique permitting evaluation of the regional variability of the metabolite concentrations in human brain. The inherent complexity of the proton spectra makes automatic methods of data analysis particularly suitable. The purpose of this work was to examine the differences between the spectra acquired from the frontal, periventricular and occipital regions exploiting two different approaches: a pattern recognition technique (partial least squares – discriminant analysis PLS-DA) applied to the full unresolved spectra and univariate analysis of metabolite concentrations output by LCModel software. The baseline correction was performed using MRUI. The data were obtained from 38 healthy volunteers (age 19-52, median age 28) using Philips Achieva 3T scanner. PLS-DA revealed the separation of the spectra belonging to different groups in the low – dimensional projection hyperplanes and enabled the regions responsible for the differences to be recognized. The latent variable associated primarily with N-acetylaspartate (NAA) and creatine (Cr) discriminated occipital and periventricular spectra from those acquired from the frontal lobe, while the difference between occipital and periventricular localizations can be mainly attributed to choline containing compounds (Cho) resonances. These findings are in accordance with the results from univariate analysis. It is also worth mentioning that due to the nature of the latent variables as linear combinations of the original features the separation between the three groups of spectra obtained from partial – least squares discriminant analysis is clearer than the discrimination offered by individual metabolite concentrations. Thus, this study supports the role of model – free pattern recognition techniques as efficient tools for classification of brain proton magnetic resonance spectra.

P022 Session 1, Thursday 1:00 - 2:30 p.m.

Monoisotopic masses (LC-nESI-LTQ-ORBITRAP) of metabolites to discriminate resistance in barley against Fusarium graminearum.

Kenchappa Kumaraswamy (1), Ajjamada Kushalappa (1), Alek (TM) Choo (2), Yves Dion (3), Sylvie Rioux (3)

(1) McGill University (2) Agriculture and Agri-Food Canada (3) CEROM

Resistance in barley (Hordeum vulgare L.) to fusarium head blight (FHB) caused by Fusarium graminearum is quantitatively inherited. Monoisotopic mass abundances of metabolites were used to discriminate barley genotype resistant (H106-4) to FHB from susceptible (H106-371). The plants were grown in greenhouse, the spikelets were spray inoculated with mock or pathogen, and covered with plastic bags to provide high relative humidity. After 48 h of inoculation, the spikelets were ground in liquid nitrogen, metabolites were extracted with methanol+water and analyzed using liquid chromatography and mass spectrometry (LC-nESI-LTQ-Orbitrap) using positive ionization and centroid data acquisition modes. Peak deconvolution and alignment were done using XCMS while peak annotation was done using ESI. A total of 3236 analytes were detected, of which1541 were retained after peak annotation and consistency across five replicates. A students t-test identified 255 metabolites with significant treatment effects. A canonical discriminant analysis of these metabolites identified constitutive resistance based on 109 metabolites that had high loading to the canonical vector 1 (CAN1) and induced resistance based on 66 metabolites that had high loading to CAN2 vector, including 27 that were common. Students t-test identified 25 resistance related constitutive and 6 resistance related induced metabolites. Students t-test and the canonical analysis together identified 166 resistance related (RR) metabolites, of which 62 were putatively assigned with compound names by matching (<10 ppm mass error) the observed monoisotopic masses with those from different databases (KNApSAcK, METLIN, KEGG, HMD, PlantCyc). Most RR metabolites belonged to phenylpropanoid (coumarin, resveratrol, dicoumarol, methyl cinnamate, safrole), flavonoid (quercetin, hypnogenol, pongapin, epigallocatechin, and alpinumisoflavone) and terpenoid (rishitin, boschnialactone, pentalenic acid, anopterine, thymoquinol, polygodial and cuauhtemone) pathways. Several of these have known antimicrobial, signaling and cell wall enforcement properties, and thus, are considered to explain the mechanism of resistance in barley against FHB.

P023 Session 2, Thursday 4:30 - 5:30 p.m.

Development of an HPLC-TOF-MS screening-platform to assess ppHypSys-dependent metabolic changes in Nicotiana attenuata during insect herbivory

Rothe Eva(1), Gaqerel Emmanuel (1), Berger Beatrice (1), Schöttner Matthias (1), Barsch Aiko (2)

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Introduction : A reliable HPLC-TOF-MS-based metabolomics platform to screen for chemical changes in leaf extracts of Nicotiana attenuata after simulated herbivory (W = mechanical wounding and OS = application of M. sexta oral secretions) is presented. This platform was used to provide insight into herbivory-induced metabolic processes depending on specific genes by comparing the responses of wild-type (WT) and genetically-transformed plants. Our initial focus was the hydroxyproline-rich glycopeptide systemin precursor (ppHypSys)-dependent metabolomic change occuring in response to the application of W+OS. After either reducing endogenous ppHypSys transcript levels by RNAi (IRsys) or over-expressing the ppHypSys precursor under the control of a 35S-promotor (OVsys), we compared metabolomic changes of WT, IRsys and OVsys in response to W+OS.

Methods: Treated and untreated leaves (control samples) from WT, IRsys, and OVsys plants were harvested 1, 4, 14, 72, 86, 120, and 168 h after treatment. The flash-frozen plant tissue was extracted with a MeOH/acetic acid buffer 40:60 (v/v). Individual extracts from three biological replicates per time-point, treatment and genotype were analyzed in duplicate by HPLC-MS using an ESI-TOF mass spectrometer in electrospray positive and negative mode. Data was evaluated using Principle Component Analysis (PCA) in the ProfileAnalysis software (Bruker Daltonik, Germany). Microsoft Office Excel was used for calculation of kinetic profiles.

Results: The total data set contained 504 individual analyses with several hundred individual peaks. The challenge was to quickly and reliably filter out the changes influenced by ppHypSys. For an initial data mining PCA was applied. The impact of specific ions on the grouping of samples, visualized by principal component analysis projections (PCA), was investigated on both a time- and genotype-scale. Quantitative information about these ions was extracted independently from statistics from the raw data files. The time-dependent concentration changes were summarized in kinetic plots. Successfully filtered ion masses were ranked and ion masses influenced by ppHypSys were sub-listed. The elemental composition of each ion of interest was calculated based on the accurate mass and isotopic pattern information. The nitrogen content can also be independently determined from wild type plants grown in N15 media. As the final goal is the structure elucidation of the relevant ions, further experiments using accurate and high resolution MS/MS data and classical NMR analysis are carried out.

Novel Aspects: Efficient data mining strategies in a large plant metabolomics data set.

P024 Session 3, Friday 4:00 - 5:30 p.m.

Metabolomics for commercial crop development

Chris Vlahakis (1), Teresa Harp (1), Jan Hazebroek (1)

(1) Pioneer Hi-Bred - A DuPont Company

A high throughput metabolomics program has been established to facilitate gene discovery and validation. Large numbers of plants are grown in highly controlled environments and many physical and chemical traits are measured. Plants are also sampled for metabolomics using regimented and well accepted protocols. Metabolomic data are acquired with GC/TOF-MS and uPLC/FTICR/MS. Very importantly, we needed to assemble and integrate project, sample, and data management software with tools that perform data alignment and normalization, as well as statistical and chemometric analyses. The result is the routine and robust ability to evaluate relationships among metabolomics "peak" or metabolite fingerprint data with metadata. Our metabolomics platform is being applied to enhance our understanding of metabolism in several important product development projects.

P025 Session 1, Thursday 1:00 - 2:30 p.m.

Response of protein and metabolite levels on nitrogen stress in Chlamydomonas reinhardtii

Do Yup Lee (1), Jeong-Jin Park (2), Oliver Fiehn (1)

(1) UC Davis Genome Center (2) Michigan State University

Metabolic transformations are subject to enzyme activity, yet most systems biology studies use transcriptomics data as surrogate for presumed downstream regulation of metabolism. While

enzyme activity is controlled by a variety of mechanisms, enzymes that exert a high degree of control over pathway fluxes ('committing steps') are often regulated by the degree of protein turnover. We here present a study that combines metabolomic and quantitative proteomic data to understand metabolic responses to environmental stress conditions.

The unicellular photosynthetic alga, Chlamydomonas reinhardtii is a model to study control of metabolism in plant-like biochemical pathways. Metabolite profiling based on GC-TOF mass spectrometry was applied to metabolic phenotyping of response in Chlamydomonas reinhardtii to environmental perturbation with gradual differences in nitrogen contents (0, 25, 50, 75 and 100% N-depletion) in a time-course study over 28 h (i.e. around two cell cycles). Even under low nitrogen stress conditions that did not result in slower growth rates or changes in chlorophyll contents, notable changes in metabolites (e.g. short chain free fatty acids) were apparent at early time points, pointing towards putative stress signaling functions of these metabolites.

In order to link biochemically coordinative mechanisms, proteomic analysis of Chlamydomonas was established by using HPLC-LTQ ion trap mass spectrometry. 517 proteins were consistently annotated in technical replicate analyses using the Tandem X and Ommsa ms/ms search engines at 99% and 95% probability (protein and peptide annotations, respectively) by Scaffold software. Among these 517 proteins, 272 were assigned as enzymes with EC numbers of which 159 enzymes were mapped to KEGG pathways concomitant with 118 identified GCTOF-metabolites. Identification of structurally yet unknown metabolites and mapping enzymes to further gene orthologs and paralogs will be used to complement annotations of the biochemical network in Chlamydomonas. Using spectral counting to assess relative abundance of proteins, differential regulation of proteins and metabolites is presented with respect to temporal changes and in response to the degree of nitrogen depletion stress.

P026 Session 2, Thursday 4:30 - 5:30 p.m.

Assessing the Molecular and Biochemical Roles of Medicago truncatula Border Cells in Plant-Microbe Interactions using Spatially Resolved Sampling and Integrated Functional Genomics

Lloyd W. Sumner (1), Bonnie S Watson (1), Ewa Urbanczyk-Wochniak (1), David V Huhman (1), Zhentian Lei (1)

(1) The Samuel Roberts Noble Foundation

The plant root tips, composed of the apical meristem and root cap, of legumes and many other plant species produce thousands of differentiated border cells that are attached to the peripheral root via water soluble mucilage. Border cells form a critical biotic boundary that is fundamental in rhizosphere modifications, ecological interactions, and plant defense. Specifically, border cells influence nodulation, induce nod genes, attract and immobilize nematodes, produce defense structures in response to fungi, bind and repel bacteria, and increase mucilage production in response to aluminum. Despite these critical roles, little is known about the molecular biology or biochemistry of these specialized and spatially resolved root cells. An integrated transcriptomics, metabolomics, and proteomics approach is being used to systematically evaluate the basal and temporal intracellular and secreted response of Medicago truncatula whole roots, root tips, and border cells to the mutualists Sinorhizobium meliloti and Glomus intraradices, and to the pathogen Phymatotrichopsis omnivora (root rot). Comparative UPLC/QtofMS metabolite profiling, which captures quantitative and qualitative information for approximately 600 components composed mostly of secondary metabolites (flavonoids, isoflavonoids, saponins, sterols, fatty acids, and conjugates of most of the preceding), has been performed and revealed substantial constitutive differences between border cells, root tips, and whole roots. Specifically, substantially elevated

levels of di, tri, and tetrahydroxy flavones and numerous unidentified compounds were observed in border. These data are further supported by microarray data that reveal the differential preprogramming and enhanced secondary metabolism and biosynthesis in border cells. Thus, it is almost certain that border cells play a more important role in plant-microbe signaling and interactions than previously realized due to elevated flavone levels. Additional experiments evaluating the metabolic reprogramming induced by P. omnivora, S. meliloti, and G. intraradices are underway. The data are revealing that border cell metabolism is significantly reprogrammed following interactions with mutualists and pathogens. Specific metabolites have been identified that are key in nodulation signaling and a new defense related compound has been identified. The details of these compounds will be presented and discussed.

P027 Session 3, Friday 4:00 - 5:30 p.m.

Analysis of metabolites in *Gochujang* using Gas Chromatography-Mass Spectrometry (GC-MS) and Principal Components Analysis (PCA)

Mi Jung Kwon (1), Sarah Lee (1), Hyung-Kyoon Choi (2), Dae-Young Kwon (3), Young-Suk Kim (1)

(1) Ewha Womans University (2) Chung-Ang University (3) Korea Food Research Institute

Gochujang is one of Korea's traditional fermented sauces, containing red peppers and soybeans, and being known to exhibit various beneficial bioactivities. Metabolites profiling was applied to discriminate Gochujang samples, fermented by different types of microorganisms, throughout 8 months of fermentation periods. Three different types of Gochujang, including traditional one and inoculated ones added with Aspergillus oryzae or Aspergillus sojae, were compared for the analysis of metabolites profiling. Diverse metabolites, including amino acids, fatty acids, and water-soluble components such as organic acids, sugars, and sugar alcohols were investigated using gas chromatography-mass spectrometry (GC-MS) and principal component analysis (PCA). A total of 21 amino acids, 12 fatty acids, 5 organic acids, 11 sugars, and 6 sugar alcohols were found in Gochujang during fermentation. In general, most of amino acids, organic acids, and sugar alcohols increased during fermentation. However, there was a significant difference in the profiling of fatty acids, particularly heptadecanoic acid, octadecanoic acid, oleic acid, and linoeic acid, in the samples with different microbial flora. In PCA, the samples taken at different fermentation times could be discriminated by the metabolic patterns of samples. The samples fermented for 0, 2, and 4 months were separated from those fermented for 6 and 8 months. In particular, asparagine, triptophan, dodecanoic acid, oleic acid, and linoleic acid were the major components that allowed the discrimination of Gochujang fermented for different periods.

P028 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolite profiling indicates a role of carbohydrate partitioning in the response of rice to salinity

Mohammad Reza Siahpoosh (1), Diego H. Sanchez (1), Joost van Dongen (1), Joachim Kopka (1)

(1) Max Planck Institute of Molecular Plant Physiology

Metabolite profiling is a fast growing technology and it is useful for phenotyping and diagnostic analyses of plants. It has been used to characterize the response of plants to a wide range of biotic and abiotic stresses. So far, the potential of metabolomics technologies to assist plant breeding and the study of genetics underlying metabolic regulation is mostly untapped. Here, we used GC-TOF-MS technology with the aim to find salt-regulated metabolites in rice seedlings (Oryza sativa). To this

end, 20 tolerant, intermediate and sensitive cultivars were profiled under three salt doses (0, 50 and 100 mM NaCl) in three independent experiments. Results showed that sugars played an important role in the metabolic phenotype, as a significant depletion of sucrose, glucose-6-phsophate and fructose-6-phosphate was detected in the root of sensitive rice cultivars under salt stress. To test whether the manipulation of sugar partitioning leads to a noticeable change in the acclimation response of rice plants to salt stress, further investigation was carried out using sucrose transporter (OsSUT1) knocked-down lines. Physiological and metabolome assessment of these antisense lines suggested that sugar maintenance in the root upon salt stress could improve the salinity tolerance of sensitive cultivars. This idea is further supported with expression data of rice sucrose transporter gene family.

P029 Session 2, Thursday 4:30 - 5:30 p.m.

Correlation between endoreduplication and metabolism in *Arabidopsis thaliana*: an LC- MS based metabolomics approach

Ruben t'Kindt (1), Lieven De Veylder (1), Kris Morreel (2), Dieter Deforce (1), Jan Van Bocxlaer (1)

(1) Ghent University (2) Interuniversity Institute for Biotechnology (VIB), Ghent University

Here we present an LC-MS based metabolomics set-up to study the role of endoreduplication in *Arabidopsis thaliana*. In the endoreduplication cycle, no mitosis and/or cytokinesis occurs between successive rounds of DNA replication. In this manner, the DNA content of the cell is doubled with every round of DNA replication, resulting in the formation of cells with a DNA ploidy level of 2C, 4C, 8C, 16C, 32C… Different hypotheses regarding the physiological function of endoreduplication are postulated. As the most common hypothesis is the link with plant growth and thus metabolic activity of the plant [1, 2, 3], a metabolomics study has been set up to investigate this theory.

To study the impact of the endoreduplication mechanism on the plant metabolome, three genotypes of *Arabidopsis thaliana* with a varying level of endoreduplication were grown. The wild-type plant (*Arabidopsis thaliana* Columbia-O) is used as a control plant with a normal endoreduplication level, while two types of transgenic plants represented a different level of endoreduplication. The CDKB1;1 genotype has a higher endoreduplication level through overproduction of a dominant negative allele of CDKB1;1 (CDKB1;1.N161), indispensable in the suppression of endoreduplication, while the $CCS52A1^{KO}$ x $DEL1^{OE}$ genotype, through crossing of a DEL1 overexpression line ($DEL1^{OE}$) with CCS52A1 knockout plants ($CCS52A1^{KO}$), shows an enhanced reduction of the endoreduplication level. DEL1 functions as an inhibitor of the endocycle [4], while CCS52 expression is linked with differentiation and endoreduplication of plant cells [5]. The endoreduplication level between these genotypes has been investigated using flowcytometric analysis.

Two batches of 20 growth plates of each genotype were harvested after 21 days of growth in a temperature controlled room. Pre-LC-MS treatment was based on homogenization with mortar and pestle and subsequent extraction with a Thermomixer using 4/1 MeOH/H_2O as the extraction solvent. The LC-MS metabolome comparison was obtained using reversed-phase (RP) micro liquid chromatography coupled with electrospray ionization quadrupole time-of-flight mass spectrometry. All plant extracts were acquired in both negative and positive ionization mode.

Multivariate analysis was applied to visualise differences between the metabolite composition of the genotypes. Principal component analysis is used to indicate systematic trends or outliers within the data. The $CCS52A1^{KO}$ x $DEL1^{OE}$ genotype shows a stronger classification, more distinct from the wild-type plant samples, compared to the CDKB1;1 genotype. Orthogonal PLS analysis corrects for

unwanted variation (i.e. the between-batch variation) between the samples and focuses on the effect of interest between the three genotypes. S-plots have been created to visualize biochemically significant metabolites differentiating between wild-type plants and transgenic plants. Both ionization modes indicate the presence of relevant differences in the metabolite pattern of the transgenic plants compared with the wild-type plant. The metabolites responsible for differentiation between genotypes are identified using MS^n analysis performed on a hybrid ion-trap/FTMS system.

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P030 Session 3, Friday 4:00 - 5:30 p.m.

Metabolic analysis of white guava fruits at different ripening stages

Sarah Lee (1), Hyung-Kyoon Choi (2), Somi Kim Cho (3), Young-Suk Kim (1)

(1) Ewha Womans University (2) Chung-Ang University (3) Cheju National University

Gas chromatography coupled with time of flight mass spectrometry (GC-TOF/MS) and multivariate statistical data analysis (MVDA) were used to study metabolites profiling of white guava (*Psidium guajava*) fruits. Both flesh and peel of ripened and unripened white guava fruits were compared for the analysis of metabolites profiling. Some amino acids and organic acids, such as alanine, serine, aspartic acid and malic acid, were major compounds to distinguish between flesh and peel of guava fruits. Also, metabolic profiling of fruits showed a significant change in some metabolites occurring during ripening. The ripened and unripened guava fruits could be separated by principal components analysis (PCA). The major components contributing to the separation were (z)-3-hexenal, proline, citric acid, malonic acid and some unknowns. In particular, valine, alanine, malic acid, citric acid and malonic acid were related to the ripening of guava fruits.

P031 Session 2, Thursday 4:30 - 5:30 p.m.

Tannins from Hamamelis Virginiana: antitumoral properties on colon cancer cells

Santiago Diaz-Moralli (1), Susana Sanchez-Tena (1), Gema Alcarraz-Vizan (1), Josep Lluis Torres (2), Marta Cascante (1)

(1) University of Barcelona (2) Institute for Chemical and Environmental Research, CSIC

Witch hazel (Hamamelis Virginiana) extracts are used in traditional medicine. Its bark is a rich source of oligomeric proanthocyanidins as well as hydrolysable tannins. Previous studies showed that galloyllation and probably the degree of polymerization influence the biological activity of tannins. We have examinated the response of human colon adenocarcinoma cells HT29 to treatment with

Witch Hazel polyphenolic fractions with different mean degree of polymerization and percentage of galloylation.

The antiproliferative properties of the fractions on HT29 cells were determined. IC50 for the different fractions were OWH 33±3 μg/mL, IIH 34±3 μg/mL, VIH 25±1 μg/mL, VIIH 57±2 μg/mL, VIII 22±2 μg/mL and IXH 27±2 μg/mL. We have investigated the influence of the fractions on the cell cycle and apoptosis observing cell cycle arrest at the S-phase and apoptosis and necrosis induction by all treatments. Furthermore we analyzed the influence of Witch Hazel extracts in HT29 cell metabolism. This findings provide a better understanding of the structure-bioactivity relationship of polyphenolics, which should help to choose the adequate source and to rationally design formulations of plant polyphenolics in nutritional supplements.

P032 Session 2, Thursday 4:30 - 5:30 p.m.

Fragilities in tumor metabolism could become new targets for novel designed anticancer therapies

Miriam Zanuy (1) Antonio Ramos-Montoya (2) Paul WN Lee (3) Josep Centelles (1) Marta Cascante (1)

(1) University of Barcelona (2) Cambridge Research Institute-University of Cambridge (3) Biomedical Research Institute at the Harbor-UCLA Medical Center, RB1

Cancer cells present a metabolic network adaptation that confer advantages to rapid proliferation, invasion and survival in hypoxic conditions. This metabolic robustness also can present points of fragility. To identify these fragilities, combination therapies based on targeting the nucleic acid synthesis metabolic network at multiple points were tested. Results showed that cancer cells overcome single hit strategies through different metabolic network adaptations demonstrating the robustness of cancer cell metabolism. When the nucleic acid synthesis inhibitors were administered individually in HT29 human colon adenocarcinoma cells, different metabolic network adaptations to overcome the inhibitory effects occurred. However, when these agents were administered in combination, the combinations exhibited either synergistic or additive effects in inhibiting cell growth, depending on the specific locations of the targeted pathways within the metabolic network. Analysis of these adaptations also identified the maintenance of pentose phosphate cycle oxidative and non-oxidative balance to be critical for cancer cell survival and vulnerable to chemotherapeutic intervention. This new principle for rational drug design originates from the integrative, systems biology approach of understanding cell function based on the operation of the nucleic acid synthesis metabolic network and opens new ways to develop novel treatments for cancer.

P033 Session 3, Friday 4:00 - 5:30 p.m.

CE-MS-based metabolomics elucidated cytotoxic mechanisms of kigamicin, a novel anticancer drug candidate

Kenjiro Kami (1), Eriko Tomitsuka (2), Yoshihiro Toya (1), Sakyo Kanehara (2), Masaru Tomita (1)

(1) Keio University (2) National Cancer Center Hospital East

Tumors typically suffer chronic hypoxic and glucose-starved stresses induced by inadequate blood supply due to structural and functional abnormalities of tumor vasculature; however, certain cancer types such as pancreatic cancer exhibit a strong tolerance against these stresses. From this perspective, a compound that exerts cytotoxic effects solely under a nutrient-free condition, such as

kigamicin, is promising as a novel-type anticancer drug that eliminates nutritionally robust malignant cells. In order to elucidate metabolic mechanisms of kigamicin toxicity on the cells, we conducted time-course metabolome analyses of Panc-1 by using capillary electrophoresis time-of-flight mass spectrometry (CE-TOFMS) under glucose-deprived condition with or without kigamicin (analysis I). We also performed this analysis after kigamicin treatment with or without glucose (analysis II) to reveal underlying mechanisms of toxicity avoidance of the cells when glucose is present. As a result, variations of 130 metabolites involved in glycolysis, the pentose-phosphate pathway, the tricarboxylic acid (TCA) and urea cycles, and amino acid, purine, and pyrimidine metabolism were obtained and comprehensively mapped on a metabolic pathway. In particular, we found a significant reduction of ATP, GTP, and most TCA-cycle intermediates under the glucose-deprived condition with kigamicin in analysis I. A metabolic flux analysis based on the distribution pattern of 13C speculated that the TCA cycle was arrested possibly due to the failure of oxidative phosphorylation under this condition. In contrast, lactate was accumulated in the medium and ATP and GTP levels were maintained if glucose is present in analysis II. Hence, it was found that kigamicin imposes devastating effects on energy metabolic machinery by presumably inhibiting a portion of electron transport chain, which thus collapses energy charge and causes inevitable necrotic cell death. However, under the presence of glucose, the cells stay alive by fully exploiting anaerobic glycolysis to maintain energy status. In conclusion, we elucidated the metabolic mechanisms of kigamicin toxicity and demonstrated that CE-MS-based metabolomics greatly facilitates the elucidation of biochemical effects of compounds functioning with unknown mechanisms.

P034 **Session 1, Thursday 1:00 - 2:30 p.m.**

Metabolic changes caused by ethinylestradiol in human breast cancer cells as observed by NMR spectroscopy[93]

Quincy Teng

A major goal of toxicity testing is to obtain toxicity data for protecting public health and the environment from adverse effects that may be caused by exposure to environmental agents in the air, water, soil and food. The current 'omic toxicological studies that target human health effects primarily rely on animal studies. Unfortunately, large-scale animal exposure studies are expensive, requiring dedicated care and handling facilities. Furthermore, the US EPA and other organizations have adopted the goal of reducing animal usage in future toxicity testing programs.

One potential approach to increase the throughput of metabolomics is to use cell cultures instead of live animals in exposure studies. Although effective extrapolation to whole organism responses is ultimately required, such an approach provides obvious advantages. For example, there is no need to house and sacrifice animals, costs are significantly lower, and cells can be grown and exposed rapidly. Also, human cell lines can be employed in order to avoid cross-species extrapolations. To conduct cell culture based metabolomics, we have developed a novel sample preparation method using adherent mammalian cells, which is rapid, effective, and exhibits greater metabolite retention by approximately a factor of 50 compared to the conventional sample preparation method.

We have applied this approach to study the metabolic changes caused by 17α-ethynylestradiol (EE2) in estrogen receptor (ER)-positive MCF-7 and ER-negative MDA-MB-231 human breast cancer cell lines using NMR spectroscopy. Details of sample preparation, NMR spectroscopy, metabolite identification, statistical analysis and intracellular metabolic changes will be presented.

P034-2 Session 1, Thursday 1:00 - 2:30 p.m.

Toxicity assessment of 17α-ethinylestradiol by cell-culture based NMR Metabolomics

Drew Ekman, Quincy Teng, Wenlin Huang, Timothy Collette

(1) Environmental Protection Agency

Small fish such as fathead minnow (*Pimephales promelas*) and zebrafish (*Danio rerio*) are widely used toxicological model vertebrates for investigating the effects of the chemicals in the environment. The changes in the metabolic profiles induced by endocrine disrupting chemicals (EDC) have the potential to be used as an effective method to assess the impacts, determine the modes of actions and identify exposure biomarkers of the specific toxicities of the EDCs. In addition to animal studies, development of metabolomics approaches that involve exposing cell cultures to potentially toxic chemicals for high-throughput screening of large inventories of chemicals has gained increasing importance.

A zebrafish liver cell line (ZFL) established from adult zebrafish has been used in a variety of biological research, including toxicology, pharmacology, developmental biology and molecular genetics. The goal of this study is to develop an *in vitro* approach to identify the responsive changes in cellular metabolic profiles induced by the exposure of chemicals. For this purpose, ZFL cells were exposed to a pharmaceutical estrogen 17α-ethinylestradiol (EE2) at two different concentrations (0.5 or 5.0 ppb) for 48 hours or 96 hours. EE2 released by some sewage treatment plants into the aquatic environment can produce diverse biological and physiological effects in exposed fish and wildlife, including degradation of reproductive and developmental behaviors. The metabolites of the cellular extracts were profiled by 1D NOESY NMR experiments. Statistical analysis showed that the perturbational metabolic profiles in the ZFL cells exposed to EE2 have both dose- and time-dependences. The results demonstrated that cell culture based metabolomics using zebrafish liver cell line can potentially be used to screen chemicals for ecotoxicity. We are currently applying the *in vitro* metabolomics approach to assess toxicity of chemicals in human cell lines. Details of sample preparation, NMR spectroscopy, metabolite identification, statistical analysis and cellular metabolite profiling will be presented.

P035 Session 2, Thursday 4:30 - 5:30 p.m.

Isotopomer Lipid Metabolomics in Cancer Cells by 2D-NMR and FTICR-MS Reveals Detailed Lipid Metabolism

Richard Higashi (1), Andrew Lane (1), Tae Hoon Yang (1), Zhengzhi Xie (1), Teresa Fan (1)

(1) University of Louisville

Turnover and composition of glycerophospholipids (GPLs) plays an important role in numerous types of cancers. Until recently, identification and quantification of PLs have been very challenging tasks for biological samples. Furthermore, to track metabolic turnover of lipids, stable isotope labeling experiments are required. However, isotope-labeling results in the need to analyze for 10-100 fold more lipids due to the synthesis of numerous mass isotopomers; these isotopomers can NOT be chromatographically resolved. Thus, a single sample can produce thousands of such GPL species to analyze, for most of which authentic standards are not, and will not be available. However, the combination of high field 2D-NMR and Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FTICR-MS) provides rapid peak assignment as well as potential for identification, resulting in extremely detailed isotopomer information. First, high field 2D-NMR provides rapid and

accurate positional isotope quantification of the glycerol and fatty acyl chains as an average, but it does not provide isotopomer speciation. On the other hand, FTICR-MS, enables the analysis of GPL mass isotopomers in complex mixtures without chromatographic separation, via direct infusion nanoelectrospray. In this approach, thousands of MS peaks are acquired in just a few minutes; a resolution >200,000 (@ m/z 400), and accuracy of better than 1 ppm (@ m/z 400) is needed for this high-information-throughput analysis. Despite the speed, extremely detailed mass isotopomer distribution of the isobaric lipids following biosynthetic labeling with [U-^{13}C]-glucose can be determined. The specific labeling patterns obtained from FTICR-MS and supported by 2D NMR were also analyzed in terms of the utilization of glucose transformations in AcCoA and glycerol, as well as the contribution from preexisting pools of unlabeled metabolites. To accomplish this, we have developed software tools to model the observed patterns in the framework a metabolic pathways which led to unprecedented detail of both intact lipid and acyl chain biosynthesis. We have applied this novel technology to lipid biosynthesis in several cancer cell lines.

P036 Session 3, Friday 4:00 - 5:30 p.m.

Validation of global metabolomic analysis in human cancer cells in culture profiling of LY294002 in U87MG glioblastoma cells

Rupinder Pandher (1), Celine Ducruix (1)

(1) Institute of Cancer Research

We have evaluated the metabolic profile in media of human cancer cells with the aim of establishing a 'fingerprint' or biomarker profile of signal transduction inhibitors.

The first aim of this study was to perform a validation of the analysis of the cell culture media in order to reliably and consistently identify specific changes in excreted or consumed metabolites. Analysis was performed on two different Q-TOFs: Agilent 6510 (S1) and Waters Premier (S2). LC separation was performed using a reverse phase Waters Acquity T3 C18 column and mass spectrometric detection was carried out using electrospray ionisation mode on both systems. We analysed 10 replicates of tissue culture media in positive and negative ionisation mode and proceeded to evaluate the number of molecular features detected and the variability of the chromatographic signal in both systems. Using the appropriate software packages, the number of variables detected were ~900 and ~1200 in positive ionisation mode and ~250 and ~400 in negative ionisation mode for S1 and S2 respectively. In positive ionisation mode, the coefficient of variations (CVs) in the integrated chromatographic signals were below 35% for 50 and 60 % of the feature identified and below 25% for 46 and 50 % of variables for S1 and S2 respectively. In negative ionisation mode, CVs were below 35% for 53 and 59 and 25% for 48 and 48 for S1 and S2 respectively. The large number of ions measured with high reproducibility in these analyses suggests that relatively small changes should be detectable in a large proportion of the metabolome in both systems.

This was confirmed by treating U87MG human glioblastoma cells with a generic PI3K inhibitor (LY294002) at two concentrations (1x and 5x cellular GI50) and comparing the metabolome of control and treated cells over time. Amongst the metabolites increased following cell treatment with 1x and 5x GI50 LY294002, m/z 496.34 was identified as glycerophosphocholine with a mass accuracy as low as 0.4 ppm and 1.4 ppm on S1 and S2 respectively. Interestingly, NMR based metabolomics showed altered choline metabolism in breast cancer cell extracts following administration of LY294002 (1)

In conclusion, this study demonstrates the overall robustness and reproducibility of our metabolomic method on both instruments and confirms that identification of specific metabolites can be achieved by this technology.

This work was supported by Cancer Research UK, The Institute of Cancer Research and the Drug Development Unit of the Royal Marsden Hospital.

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P037 Session 1, Thursday 1:00 - 2:30 p.m.

Anticancer Methylseleninic Acid Disrupts Metabolic Pathways in Human Breast Cancer Cells Discerned by 13C-Isotopomer Profiling

Teresa Fan (1), Zhengzhi Xie (1), Tae Hoon Yang (1), Richard Higashi (1), Andrew Lane (1)

(1) University of Louisville

Breast cancer is the leading cancer among women and its incidence is approximately one in eight. About two thirds of breast cancer cases are estrogen (E2)-sensitive and can benefit from anti-estrogen (e.g. tamoxifen or TAM) adjuvant therapy. However, a significant fraction of E2-sensitive breast cancers, metastatic forms in particular, ultimately develops resistance to TAM [1]. Overcoming this problem is vital to breast cancer treatment. We recently found that a TAM-resistant variant of MCF7 breast cancer cell line, LCC2, was very sensitive to an anti-cancer selenium agent, methylseleninic acid (MSA) at low μM range. To elucidate the molecular mechanism for this sensitivity, we employed uniformly 13C-labeled glucose as a tracer to profile time-dependent perturbations to metabolic networks in MSA-treated LCC2 cells. NMR and GC-MS were used to profile polar metabolites and their 13C-isotopomers. FT-ICR-MS in conjunction with in-house PRecalculated Exact Mass Isotopomer Search Engine (PREMISE) was employed to assign phospholipids (PL) and their 13C-isotopomers. Relative to the control, many polar metabolites (lactate, amino acids, Krebs cycle metabolites, glutathione) showed a transient buildup at 6 hr in MSA-treated LCC2 cells. Some other polar metabolites (phosphocholine, adenine nucleotides, and NAD+) also peaked at 6 hr but depleted thereafter up to 24 hr. Many of these changes were accountable by the corresponding changes in 13C-labeled metabolites, but the large buildup of unlabeled Gln was not accompanied by its increased synthesis from glucose. These data indicate perturbations to glycolysis, Krebs cycle, and Gln uptake and/or oxidation. Synthesis of acyl moieties of PLs was also disrupted by MSA treatment in LCC2 cells, as revealed by both NMR and FT-ICR-MS analysis; the latter provided the relative abundance of many different PL and their 13C mass isotopomers. Among the different classes of PL, the 13C enrichment in individual mass isotopomers of phosphatidylcholine with R1=C16:1 and R2=C18:0 was prominent, which allowed computation of precursor labeling, thereby enabling quantitative analysis of lipid biosynthetic pathways perturbed by MSA.

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P038 Session 2, Thursday 4:30 - 5:30 p.m.

1H-NMR based Metabonomics Investigation of Rheumatoid Arthritis in humans: Identification of Biomarkers and Monitoring Progress of Treatment and Disease Severity.

Michael Lauridsen (1), Henning Bliddal (2), Bente Danneskiold-Samsoe (2), Mikkel Helleberg Dorff (3), Hector Keun (4)

(1) Novo Nordisk A/S (2) Frederiksberg University Hospital (3) skilde Hospital (4) University of London

Objective: Rheumatoid arthritis (RA) is a chronic inflammatory condition associated with a prevalence of 1% and implies a significant reduction in quality of life for patients. A cure is currently not available; treatment is palliative and at best slowing down disease. Over the past decade, therapy has been dramatically changed by the introduction of effective biological products, most prominently anti-TNF-alpha;products. The aim of this study was to test patients with active RA before and during anti-TNF-alpha; therapy, patients in close-to-remission on anti-TNF for the full metabolic profile as a basis for possible future evaluation of candidate biomarkers for disease severity and treatment progress.

Methods: 1H-NMR spectroscopy of human plasma in combination with orthogonal projection to latent structures discriminant analysis (O-PLS-DA) was applied. 25 patients with active disease (joint inflammation) were enrolled and tested at the time of inclusion (t = 0) before biological therapy was initiated and after t = 14, 31, 182 and 365 days during therapy. A second patient group included 25 patients in remission at t = 0, 182 and 365 days all treated with anti-TNF-alpha; treatment regimes. Furthermore, 50 healthy controls were enrolled (t = 0, 182 and 365 days). All samples were drawn in the morning after fasting from midnight.

Results: O-PLS-DA showed good discrimination (Q2 = 0.5) between patients with active RA and healthy controls, proposing lactate, N-acetyl glycoprotein and HDL as the most interesting candidate biomarkers. The biochemical profile associated with RA enabled verification of the already existing link between RA and coronary artery disease. ANOVA and multiple comparison tests showed that the metabolic profile of patients with active RA, as a consequence of optimized treatment, approached the metabolic profile of patients in remission. However, the metabolic profiles of both patient groups were different from the healthy controls.

Conclusion: 1H-NMR spectroscopy seems promising in the effort to discriminate whole-body metabolism in RA regarding sick vs. healthy as well as active vs. inactive disease.

P039 Session 3, Friday 4:00 - 5:30 p.m.

Metabolomics based investigations of Rheumatoid Arthritis

Rasmus Madsen (1) , Thomas Moritz (2) , Jon Gabrielsson (3) , Gunillla Ekström (4) , Torbjörn Lundstedt (5)

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The lecture will go through the results from several independent, but very much related studies on the disease Rheumatoid Arthritis (RA). RA is a serious auto-immune disease, that mainly affects smaller joints trough swallowing and pain, but the patients also carries increased risk of lethal cardiac episodes[1]. From a physicians viewpoint the disease is problematic since both diagnosis and

treatment is non-trivial. In early stages the disease can appear similar to other joint diseases and though halt proper treatment. The possible treatments have widely varying results in different patients and the process is often one of trial and error.

In order to improve the care we have used metabolomics techniques such as mass spectrometry and multivariate data analysis to find new ways of diagnosing the disease as well as following the treatment. We have shown that the blood metabolite patterns of RA patients can be used to distinguish them from healthy subjects, but also from patients with clinically similar diseases such as Osteoarthritis (OA) and Psoriatic Arthritis (PsA). The discovered metabolite patterns have revealed some interesting characteristics that can possibly be useful when following patient care.

Furthermore we have used the techniques for evaluating suitable model systems for drug development for RA. The metabolite patterns found in human RA was to some degree also distinguishable in common animal models of RA. Different treatments have been assessed in both animals and humans, by studying the effects they have on the metabolite patterns and comparing these changes to healthy and diseased subjects respectively.

The investigations have utilized both GC-TOF based metabolomics and more specific metabolite profiling using LC-MS. Most models have been using orthogonal projection to latent structures[2] (OPLS) since this technique provides easily interpretable models and therefore increases the biological knowledge derived from metabolomics studies.

1. Firestein, G.S. Evolving concepts of rheumatoid arthritis. Nature 423, 356-361 (2003).
2. J. Trygg, S.Wold Orthogonal projections to latent structures (O-PLS). Journal of Chemometrics 16, 119-128 (2002).

P040 Session 1, Thursday 1:00 - 2:30 p.m.

Altered interactions of tryptophan metabolites in first-episode neuroleptic-naïve patients with schizophrenia

Jeffrey Yao (1) , George Dougherty (1) , Ravinder Reddy (2) , Matcheri Keshavan (3) , Debra Montrose (2)

(1) VA Pittsburgh Healthcare System (2) University of Pittsburgh (3) Wayne State University

Schizophrenia is a biologically complex disorder with perturbations in multiple neurochemical systems whose dynamic relations, until recently, has been difficult to examine. Rather, evidence for these alterations has been collected piecemeal, limiting our understanding of the interactions amongst relevant biological systems. Previously, both hyper- and hyposerotonemia were associated with clinical course of chronic illness in schizophrenia, suggesting a progressive disturbance in the central serotonin (5-HT) function. Using a targeted electrochemistry based metabolomics platform, we have identified metabolic signatures consisting of 13 tryptophan-metabolism products simultaneously in the plasma between first-episode neuroleptic-naive patients with schizophrenia (FENNS, n=25) and healthy controls (HC, n=30) as well as between FENNS at baseline (BL) and 4 weeks (4w) after antipsychotic treatment. The Wilcoxon rank-sum tests showed higher N-acetylserotonin in FENNS-BL compared to HC ($p = 0.0077$, a "trend-level" difference after Bonferroni correction of an alpha of 0.05 to 0.0038). Moreover, N-acetylserotonin/tryptophan and melatonin/serotonin ratios were higher, and melatonin/N-acetylserotonin ratio was lower in FENNS-BL (all p values<0.0029), but not after treatment, compared to HC subjects. On the other hand, all 3 groups had highly significant correlations between tryptophan and its metabolites, melatonin, kynurenine, 3-hydroxykynurenine and tryptamine. Contrarily, in the HC but not either FENNS groups, serotonin was highly correlated with tryptophan, melatonin, kynurenine, or tryptamine, and 5-HIAA was highly correlated with tryptophan, melatonin, kynurenine, or 3-hydroxykynurenine. Using

simultaneous Šidák 95% confidence intervals (Larntz-Perlman) with non-null multiple correlations across the groups, a significant difference between HC and FENNS-BL was further demonstrated only for the tryptophan-5HIAA correlation. Taken together, some metabolite interactions within the tryptophan pathway appear to be altered in FENNS-BL patients. Specifically, conversion of serotonin to N-acetylserotonin by serotonin N-acetyltransferase was up-regulated in FENNS patients, possibly related to an altered Trp-5HIAA correlation. Considering N-acetylserotonin as a potent antioxidant, such increases in N-acetylserotonin might be a compensatory response to increased oxidative stress in schizophrenia.

P041 Session 2, Thursday 4:30 - 5:30 p.m.

Impaired Antioxidant Defense System in First-Episode Neuroleptic-Naïve Patients with Schizophrenia

Jeffrey Yao (1), George Dougherty (1), Matcheri Keshavan (2), Debra Montrose (3), Wayne Matson (4)

(1) VA Pittsburgh Healthcare System (2) Wayne State University (3) University of Pittsburgh (4) Bedford VA Medical Center

We have previously demonstrated reduced plasma uric acid levels in first-episode neuroleptic-naive patients with schizophrenia (FENNS). Uric acid is an end-product of purine pathway, and has been shown to have free radical scavenging properties. Thus, purine catabolism may contribute to mitochondrial antioxidant defense system (AODS) resulting from the production of urate. Using high-pressure liquid chromatography coupled with a coulometric multi-electrode array system, we compared 6 purine metabolites simultaneously in plasma between FENNS (n=25) and healthy controls (HC, n=30) as well as between FENNS at baseline (BL) and 4 weeks (4w) after antipsychotic treatment. Wilcoxon rank-sum tests showed significantly higher levels of xanthosine and lower levels of guanine in both patient groups compared to HC subjects. Moreover, xanthosine/guanosine and xanthosine/guanine ratios were higher, and urate/xanthosine ratio was lower in both patient groups (all p values<0.008) compared to HC subjects. On the other hand, all 3 groups had highly significant correlations between urate and guanine or between hypoxanthine and xanthine. Contrarily, in the HC but not either FENNS groups urate was highly correlated with xanthine or hypoxanthine. During purine catabolism, both conversions from guanosine to guanine and from xanthosine to xanthine are reversible. Decreased ratios of product to precursor suggested a shift favorable to the precursor in the FENNS. Taken together, the potential for steady formation of antioxidant urate from purine is altered early in the course of illness and independent of treatment effects. The present data provide further support of a defect in the AODS in schizophrenia.

P042 Session 3, Friday 4:00 - 5:30 p.m.

GC-MS analysis of cerebrospinal fluid (CSF) to search for biomarkers for CNS disorders

Leon Coulier

TNO Quality of Life

CNS disorders are among the most important health problems of today from a medical and social-economic perspective. Current drug treatment in CNS disorders (if available) mainly focus on suppression of symptoms rather than cure. Cerebrospinal fluid (CSF) can be considered to be the best compartment for discovery and pharmacokinetics/ pharmacodynamics (PK/PD) modeling of

biomarkers that predict drug related exposure and efficacy in CNS disorders. Despite its potential, little optimization of use of CSF for biomarker research, especially metabolomics, has been done. Top Institute Pharma in the Netherlands has initiated a project aiming at professionalizing the use of CSF for biomarker discovery and analysis. In this paper the focus will be on the use of GC-MS for CSF metabolomics. Issues that will be addressed are coverage, biological vs. analytical variation, reduction of sample volume, data-preprocessing and application to clinical studies. It will be shown that GC-MS is a suitable metabolomics method to study CNS disorders using CSF.

P043 Session 1, Thursday 1:00 - 2:30 p.m.

MRS and Pattern Recognition in Central Nervous System Disorders

Maria Sokol (1), Ewa Jamroz (2), Justyna Paprocka (2), Magdalena Wicher (3)

(1) Institute of Oncology (2) Silesian Medical University (3) Helimed

Background: CNS disorders are widely analyzed using MRS. However, there is a lack of models describing metabolic profiles of particular disorder types suited for diagnosis support. We made an attempt to create such models and use them for automatic differentiation of several CNS disorders.

Methods: 19 patients with confirmed central nervous system disorders divided into: inflammatory / demyelinating disease (ID), neurometabolic disease (NM) and cerebral palsy (CP) groups were subjected to the examination. 77 proton spectra were obtained using MRI/MRS system operating at the proton resonance frequency of 81.3 MHz. In order to visualize the metabolic differences between studied groups as well as in-group - origin depending differences, Partial Least Squares Discriminant Analysis (PLS-DA) with orthogonal signal correction (OSC) spectral filtering was applied to unresolved spectra.

Findings: Created model efficiently distinguished the studied groups. Significantly elevated Lip, Lac and Ala signals (0.82-1.62 ppm) in NM and increased mI and Glx (3.63 and 3.9 ppm) levels in CP distinguish both the groups. Moreover both, above-mentioned groups showed significantly lower NAA (1.95-2.03 ppm) signals than the ID group. Detailed analyses of particular groups revealed the strong influence of mitochondrial disorders on Lip and Lac elevation in NM group. Among ID patients, the highest NAA signals were observed in CNS inflammation, while increase of Lac/Lip signals and a slight decrease of Cho and NAA levels in demyelination neuropathy.

Interpretation: The use of MRS coupled with PR methods allows building diagnostically helpful models able to distinguish between several disease types in the same time.

P044 Session 2, Thursday 4:30 - 5:30 p.m.

Potential Effects of Gamma-Tocopherol in the Immune Set Point could Influence the Antidepressant effects of Lithium.

Marlon Quinones (1), Oliver Fiehn (2), Jair Soares (1), Rima Kaddurah-Daouk (3)

(1) University of North Carolina at Chapel Hill (2) UC Davis (3) Duke University

Background: The power of metabolomics was used to identify potential biochemical markers of treatment response in bipolar disorder. Baseline levels of Gamma-tocopherol, a lipid soluble antioxidant/anti-inflammatory isoform of Vitamin E that plays a regulatory role in monoamine

metabolism, were negatively correlated with Lithium's anti-depressant response. Here we focused on defining potential mechanisms underling this observation. Gamma-tocopherol is an inhibitor of cyclooxygenase thought to have immunomodulatory effects. Mounting amount of evidence suggests that immune dysregulation might contribute to the pathogenesis of mood disorders. Moreover, Lithium has powerful effects on the immune response. Thus, we hypothesized that gamma-tocopherol levels might be linked to different immunological set points (as manifested by circulating cytokine levels); and that in the context of a high immune set point (linked to low gamma-tocopherol levels), the antidepressant effects of Lithium might become more evident.

Methods: Individuals with DSM-IV Bipolar Disorder Type I (n=11, age= 27±4.7yo, 80% females and medication-free for at least 2weeks) experiencing a depressive or mixed episode received daily treatment with Lithium for 4weeks. During this time, the dose of Lithium was tailored to reach therapeutic blood levels between 8-1.2 mEq/L. Trained raters assessed mood symptoms before and after treatment using the Hamilton Depression Rating Scale (HAMD). The magnitude of response was defined as percentage reduction in mood ratings. Blood samples were collected at the time of mood assessment, plasma isolated and frozen right away. Metabolomic and immunological assessment were conducted using GC-TOF mass spectrometry in conjunction with BinBase data annotation and multiplex immunoassay (RBM, Austin), respectively. Analyses were done on normalized data. For partial correlations, age and sex were used as covariates (significance= uncorrected P<0.05).

Results: Lithium-induced reduction on depressive symptoms was significantly correlated with pre-treatment levels of gamma-tocopherol (*r*=-0.81, *P*=0.004). Providing support to the notion that gamma-tocopherol immunomodulatory effects might influence the immune set point, there were significantly negative correlations between baseline levels of Gamma-tocopherol and the mediators, interleukin (IL)-5 (*r*=-0.69, *P*= 0.02), IL-10 (*r*=-0.67, *P*= 0.02), IL-12 (*r*=-0.58, *P*= 0.01), IL-13 (*r*=-0.78, *P=0.0009*) and IL-15 (*r*=-0.59, *P=0.02*). With the exception of IL-5, levels of all these mediators were positively and significantly correlated with reductions in HAMD scores seen with Lithium treatment (IL-10 *r*=0.64, *P*= 0.04; IL-12 *r*=0.55, *P*= 0.04; IL-13 *r*=0.75, *P=0.01*; and IL-15 *r*=0.57, *P=0.04*), suggesting that indeed, a high immune set point (elevated plasma cytokines) might be related to higher Lithium responsiveness. Levels of Gamma-tocopherol or immune factors were not significantly correlated with pre-treatment HAMD scores ruling out the possibility that severity of the illness alone could account for our findings. The specificity of association between Gamma-tocopherol and immune set point was emphasized by the observation that Octadecanol levels were correlated with a reduction in HAMD scores (a fatty alcohol; *r*=0.82*, P*=0.006) but not to any of the immune factors studied.

Conclusion: Gamma-tocopherol levels and pre-treatment immune set point might influence the magnitude of antidepressant response to Lithium in bipolar illness. Replication studies are warranted.

P045 Session 3, Friday 4:00 - 5:30 p.m.

Differential Effects of Atypical Antipsychotics on Inflammatory Cytokines Detectable Early in the Treatment of Schizophrenia: Possible Implications for the Development of Metabolic Side Effects

Marlon Quinones (1) , Rebecca Baillie (2) , Katie Jeffress (3) , Lauren Bayer (3), Joseph McEvoy (3)

(1) University of North Carolina at Chapel Hill (2) Rosa & Co LLC (3) Duke University

Clear differences in effectiveness and side effects profile have been reported among Olanzapine, Risperidone and Aripiprazole. For instance, while being among the most clinically effective of the

three, Olanzapine, also carries the highest risk of inducing metabolic abnormalities such as insulin resistance. Given that chronic inflammation is thought to contribute to the development of metabolic abnormalities; and increasing evidence suggest that Inflammation might play a role in the pathogenesis of Schizophrenia, we asked whether treatment with Aripiprazole, Risperidone or Olanzapine had any effect on plasma levels of inflammatory mediators (cytokines). We focused on the cytokines Tumor Necrosis Factor (TNF)α and Interleukin (IL)-6 for the following reasons: 1) There is an overwhelming amount of evidence linking elevations in TNFα levels to the development of insulin resistance; 2) both cytokines have consistently been shown to be elevated in schizophrenics compared with controls; and, 3) Treatment with Clozapine, another atypical antipsychotic, was shown to increase plasma levels of TNFα.

Methods: Adult male patients with DSM-IV schizophrenia (with no antipsychotic treatment for at least 3 weeks prior to admission) were divided into three treatment groups receiving 10-30 mg/day of Olanzapine (n=5); 2-6 mg/day of Risperidone (n=10); or 10-15 mg/day of Aripiprazole (n=3). Plasma was isolated from pre- and 2-3 weeks post-treatment morning blood samples, frozen within 10 minutes of collection at -80^{o}C and thaw immediately prior to assay cytokines. A multiplex bead array ELISA assay was used for quantification of the cytokines TNFα and IL-6 (LINCOplex assay, Linco Research). Clinical Global Impressions (CGI) Scale at baseline; and at the time of follow-up blood draws was used for Clinical assessment. Pre- vs. post-treatment cytokine levels were compared using Wilcoxon ranking followed by paired t-test. Other comparisons were conducted using ANOVA. Significance= $P<0.05$.

Results: There were no significant differences in age, weight or severity of the illness (baseline CGI scores) across treatment groups. Treatment was associated with significant clinical improvement (CGI change) in each treatment group. There were no significant differences in mean CGI change between treatment groups. A significant increase in post-treatment TNFα plasma levels as compared to pretreatment was seen in the Olanzapine group ($P=0.02$); but not in the Risperidone or Aripiprazol groups. There were not significant differences in IL-6 levels before and after treatment in any of three treatment groups.

Interpretation: An early elevation in the levels of TNFα was seen in patients treated with Olanzapine but not Risperidone or Aripiprazol. Replication studies in larger cohorts are required to determine whether induction of TNFα might be one of the mechanisms by which Olanzapine increases the susceptibility to metabolic abnormalities in patients with schizophrenia.

P046 Session 1, Thursday 1:00 - 2:30 p.m.

Abnormalities in Inflammatory Lipid Levels in First Episode Schizophrenic Patients are Modulated by Risperidone

Marlon Quinones (1), Rebecca Baillie (2), Katie Jeffress (3), Lauren Bayer (3), Joseph McEvoy (3)

(1) University of North Carolina at Chapel Hill (2) Rosa & Co LLC (3) Duke University

Background: Bioactive lipids may play a role in the pathogenesis of Schizophrenia. Inflammatory lipids are derived from the enzymatic oxidation of PUFAs (e.g., arachidonic acid) in metabolic cascades driven by cyclooxygenases (COX), leading to the generation of prostaglandins (PG), prostacyclins and thromboxanes; and by lipoxygenases, generating leukotrienes as well as monohydroxylated metabolites [e.g., 15-hydroxyeicosapentaenoic acid (HEPE)]. Reports have shown elevations in circulating levels of PGE2 in chronic schizophrenic patients; and that COX-2 inhibition potentiates Risperidone's antipsychotic effects. Here in the first study of its kind, we conducted a survey of bioactive lipids in first episode schizophrenic patients before and after treatment with Risperidone; and a demographically matched healthy control group (HC).

Methods: Subjects included 6 male patients with DSM-IV schizophrenia (first psychotic episode with no antipsychotic treatment for at least 3 weeks prior to admission) and 6 demographically matched HC. After overnight fasting, morning blood samples were drawn from HC and patients (pre- and 2-3 weeks post-treatment with 2-6 mg/day of Risperidone). Plasma was isolated within 10 min and frozen at -80^{o}C. Immediately prior to analysis, samples were thawed, spiked with internal standards and extracted by solid phase extraction using Oasis HLB cartridges (Waters Corporation, Milford, MA). The cartridges are eluted with ethyl acetate; the extracts are dried under nitrogen, and re-dissolved in methanol. The samples are analyzed using HPLC separation, negative mode electrospray ionization, and tandem mass spectrometry. Clinical Global Impressions (CGI) Scale at baseline and at the time of follow-up blood draws was used for Clinical assessment. An overall global change rating was made post-treatment. For analysis we used ANCOVA (HC vs. patients pre- and post-treatment) and partial correlations (CGI change and lipid levels) with age as covariate; as well as paired samples t-test (patients pre- vs. post-treatment) with prior Wilcoxon ranking for abnormally distributed data. Significance= $P<0.05$.

Results: Treatment with Risperidone was associated with significant improvements in CGI scores. No differences were observed between patients and HC in the levels of the prostaglandins B2, D2, E2, F2, J2; Thromboxane-B2; or Leukotrienes B4, B5, C4, D4, E4, F4. Interestingly, we found that compared with HC, levels of 12- (P=0.01) and 15-HEPE (P=0.009) were significantly reduced in patients before treatment; and that after treatment, their levels increased with the difference between HC and patients remaining significant only for 15-HEPE (P=0.03). Treatment was associated with reductions on the levels of PGE2 (P=0.01) and PGD2 (P=0.02). Significant correlations between CGI change and baseline bioactive lipids levels were seen only for the metabolites 11bPGF2a (r=-.88, P=0.004) and 15 keto PGF2a (r= -.88 P=0.04), that are related to the pro-inflammatory mediators, PGD2 and PGF2a, respectively.

Interpretation:15-HEPE has been shown to have anti-inflammatory effects; lower levels might contribute to the increased inflammatory state seen in Schizophrenia. Risperidone-associated reduction in levels of inflammatory PGs might contribute to its anti-psychotic effects. Replication studies are warranted to determine whether baseline 11bPGF2a and 15 keto PGF2a levels can be useful predictors of treatment response.

P047 Session 2, Thursday 4:30 - 5:30 p.m.

Can metabolic markers in blood serum be found for distinguishing anti-epileptic drug responders from non-responders using a metabonomic approach?

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One third of all epilepsy patients are resistant to treatment with antiepileptic drugs (AEDs). In a metabonomics approach designed to interrogate this idiosyncrasy, pre-treatment blood serum samples collected from one hundred and twenty five newly diagnosed epilepsy patients, were analysed by NMR and low resolution LC-MS in combination with multivariate statistical analyses and related approaches including molecule target analysis. Treatment response (seizure-free, not seizure-free, unclassifiable) was determined by blinded review of clinical case notes and seizure diaries and assessed alongside all experimental data at six weeks, six months, and twelve months

after commencing drug therapy. Clustering within the multivariate statistical results did not map to the clinical data. A targeted metabolite search using high resolution Fourier transform LC-MS to analyse the samples. Analysis of the data using Sieve 1.1 did not reveal any clear markers. However, it was possible to discern some differences between the groups using a targeted analysis of the data. In conclusion, automated data handling of high resolution FT-MS data still requires optimisation in order to extract low level marker compounds.

P048 Session 3, Friday 4:00 - 5:30 p.m.

A metabolomic comparison of mouse models of Neuronal Ceroid Lipofuscinoses

Reza M Salek (1), Michael Pears (1), Jonathan Cooper (2), Hannah Mitchison (3), Russell Mortishire-Smith (4)

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The Neuronal Ceroid Lipofuscinoses (NCL) are a group of fatal inherited neurodegenerative diseases in humans distinguished by a common clinical pathology, characteristic storage body accumulation in cells and gross brain atrophy. We have investigated metabolic changes in 2 mouse models of NCL to examine metabolic profile of each model and compare to the existing Cln3 (Batten disease) mouse model [1]. The first model, a naturally occurring mouse mutant, termed motor neuron degeneration (mnd) mouse contains a one base pair insertion in the orthologous mouse Cln8 gene (82% identical to human gene), on a C57BL/6J background, exhibiting abnormalities akin to those in human NCL patients. The second mouse variant models late infantile NCL and is termed the neuronal ceroid lipofuscinosis (nclf) mouse. The nclf mouse contains a one base pair insertion in the orthologous mouse Cln6 gene (90 % identical to human gene) resulting in a frameshift defect. Similar to mnd mice, nclf mice on a C57BL/6J background, exhibit a characteristic accumulation of autofluorescent lipopigment in neuronal and non neuronal cells.

Brain tissues from mnd, nclf, and C57BL/6J controls, of various ages (1, 2, and 6 months) were studied using a chloroform/methanol extraction method prior to analysis by 1H NMR spectroscopy. All the metabolites were assigned using the existing literature, 2D NMR spectroscopy assisted by STOCSY analysis [2]. The NMR profiles derived from mnd and nclf mice were distinguished according to disease/wild type status in the cortex, cerebellum. In particular, glutamine was increased and gamma-amino butyric acid (GABA) decreased in the cortices of mnd (adolescent mice) and nclf mice relative to wild type in all ages of mice examined. In younger mice phosphocholine and myo-inositol were increased in the cortex of mnd mice and N-acetyl-L-aspartate (NAA) concentration was decreased; in nclf mice the contrary was observed. Comparison of our result to the existing Cln3 mice model (with similar ages ranges) showed that the metabolism of mnd mice resembled older Cln3 mice, where the disease is relatively advanced, while the metabolism of nclf mice was more akin to younger Cln3 mice, where the disease is in its early stages of progression. Our results allowed us to identify metabolic traits common to all NCL subtypes. Similar analyses should prove invaluable in understanding more of the underlying disease pathogenesis.

1. Pears, M.R et al., High resolution ^{1}H NMR-based metabolomics indicates a neurotransmitter cycling deficit in cerebral tissue from a mouse model of Batten disease. J Biol Chem, 2005. 280(52): p. 42508-14.

2. Cloarec O. et al, (2005) Evaluation of the orthogonal projection on latent structure model limitations caused by chemical shift variability and improved visualization of biomarker changes in ^{1}H NMR spectroscopic metabonomic studies. Anal Chem 77, 517-526.

P049 Session 1, Thursday 1:00 - 2:30 p.m.

Pharmacometabolomics research network for drug response phenotypes

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The Pharmacometabolomics Research Network is an NIH funded initiative that aims to "integrate" metabolomics and pharmacogenomics. Specifically, it aims to test the hypothesis that the application of metabolomics analyses and the inclusion of metabolomics data would significantly enhance pharmacogenomic research by providing broad-based, biochemically precise phenotypes capable of supplementing and extending the clinical phenotypes currently used as pharmacogenomic "endpoints". In addition, metabolomic "signatures" present in patients who do and do not respond to drug therapy, i.e., signatures that reflect the drug response phenotype, could lead to mechanistic hypotheses that would provide insight into the underlying basis for individual variation in drug response. We will present early findings from the study of two drugs simvastatin and escitalopram representatives of two major classes of drugs statins and SSRIs.

P050 Session 2, Thursday 4:30 - 5:30 p.m.

Fully unsupervised automatic assignment and annotation of sum formulae for product ion peaks, neutral losses in MS and production spectra

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An essential component on the process of characterizing chemical unknowns, is the inspection of collisionally induced dissociation (CID) mass spectra from quadrupole/time-of-flight (Q-TOF) instruments. The high resolution and superior mass accuracy is key to establish the elemental composition of molecules. Unfortunately with increasing m/z values the number of possible sum formulae grows exponentially. Several techniques have been developed which use the information from MS/MS spectra as additional criterion for reducing the number of possible formulae for the precursor ion, by summing up the potential formulae for product ion and neutral loss to establish the identity of the precursor ion. Herein we present a novel software module which implements a different route, which is exemplified with the medium sized compound Paclitaxel.

Methods: Paclitaxel was dissolved at approximately 1 mg/mL in methanol and diluted to 1 ng/microL in acetonitrile/water containing 0.1% formic acid. Infusion was performed with a syringe pump at a flow rate of 3 microL/min. Mass spectra were acquired with a micrOTOF-Q ESI-Qq-TOF mass spectrometer in ESI positive mode in scan range from 50-1000 m/z. Measurements were performed in MS full scan mode, MS/MS and IS-CID MS3. Mass spectra from all modes were externally recalibrated on MS full scan spectra. Data evaluation was performed using a software tool for the automated generation of molecular formulae from accurate MS, MS/MS, and IS-CID MS3 data.

Preliminary Results: In structure elucidation, the confident determination of the molecular formula of the parent ion is the initial step to interpretation. In the next step, MS/MS and also MS3 are important for generation of fragmentation pathways. The IS-CID in the Qq-TOF MS is achieved between two ion funnels in the high pressure region of the ion source. When doing IS-CID in the funnel and subsequent isolation and fragmentation of a first generation product ion, MS3

experiments can be performed easily in a Qq-TOF MS. In this entire evaluation process, the generation of formulae is the initial step towards interpretation. Formula generation is supported by chemical knowledge (nitrogen rule, rings and double bonds, H/C ratio) and by fitting the measured isotopic pattern (relative ion abundances, RIAs) against the theoretical one. Unfortunately the number of possible formulae grows exponentially with increasing m/z values. This can be circumvented by using MS/MS and if available IS-CID-MS3 spectra, as for the product ions the sum formula can often be assigned unequivocally, especially for those with a low m/z value. Furthermore, neutral losses are used to crosscheck the relationship of precursor and product ions. User interaction is allowed to delete wrong formulae due to knowledge of the chemical context of the sample. For the complete series of formulae from precursor and product ions, a combined quality value is calculated. The algorithm has been exemplified on measurements of Paclitaxel, whose fragmentation is well understood. Generation of formulae based solely on accurate mass with a rather conservative tolerance of m/z 0.004 and on RIAs results in 34 possible candidates, compared to four candidates from our new approach.

P051 Session 3, Friday 4:00 - 5:30 p.m.

Analyzing Metabolomics Data for for Automated Prediction of Underlying Biological Mechanisms

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(1) Case Western Reserve University

With the recent advances in experimental technologies such as gas chromatography and mass spectrometry, the number of metabolites that can be measured in biofluid samples has been increasing at a fast rate. In order to identify the metabolic mechanisms that lead to changes in concentrations of given metabolites, one needs to interpret the metabolic significance of the observed changes. This necessitates a time consuming, extensive and manual cross-referencing of metabolic pathways, in order to critically evaluate the data. The large number and breath of the metabolites represents a challenge to an informed interpretation of the results, with the goal of figuring out what possibly may have happened before the observed changes have taken place. Hence, there is a need for computational tools to help biologists and clinical researchers to derive meaningful interpretations of metabolomics data.

This paper proposes and evaluates techniques for automated interpretation and analysis of metabolomics data via the existing metabolic networks. Given a set of observations on metabolite concentration level changes, the goal of our study is to employ automated reasoning, and provide researchers with possible metabolic action scenarios that may have been implemented by the body to produce the observed metabolite changes. We start by redefining the notion of hypothesis in our context as a set of statements on concentration level changes of metabolites, created to explain a possible biological mechanism leading to the observed metabolite level changes. Hypothesis generation has four steps, the first two of which are

1. Chase Process computationally generates a set of hypotheses by chasing the observed concentration level increases and decreases within the human metabolic network. In this chase process, we employ (multiple variations of) the following metabolic reasoning: If the concentration level of a metabolite m is observed to decrease after a perturbation, then either it is consumed more and/or produced less than it was before the perturbation. Similarly, if the concentration level of a metabolite m is observed to increase after a perturbation, then either it is consumed less and/or produced more than it was before the perturbation.

2. Derived metabolite concentration level changes are obtained from (a) the observed metabolite concentration level changes and (b) the whole metabolic network.

3. Likelihood of hypothesis. We evaluate the likelihood of each hypothesis on the basis of flux ratio information and/or metabolic network topology-based information

4. Physiological condition mapping. We map each hypothesis to a (set of) physiological condition(s) based on the overlap between currently known biomarkers associated with each physiological condition and the set of metabolites that are included in a hypothesis.

We show through several case studies within the context of non-alcoholic fatty liver disease that the proposed system can generate hypotheses which are consistent with the manually created interpretations by researchers. In addition, our system can provide more detailed etiology descriptions based on the underlying metabolic network. Furthermore, our empirical evaluations show that (a) through consistency analysis against a small number of measured metabolite concentration changes, over 90% of the automatically generated hypotheses can be invalidated with no manual analysis, (b) using the proposed summarization techniques, the entire hypothesis set can be represented by a much smaller (2% of the original) hypothesis set, and (c) performing hypothesis generation and consistency checking in an interleaved manner leads to over 95% improvement in running time.

P052 Session 1, Thursday 1:00 - 2:30 p.m.

Integration of instrumental and clinical metadata for the HUSERMET project via a web service framework

Andy Tseng (1), Giles Velarde (1), Irena Spasi (1), David Broadhurst (1), Warwick Dunn (1)

(1) The University of Manchester

The metabolome, being 'downstream' of both the genome and the proteome, represents theoptimal level for understanding and predicting changes in the biology of a complex system. It isalso rapidly becoming one of the cornerstones of functional genomics and systems biology. Numerous technologies now exist for measuring the metabolome, primarily mass spectrometrylinked to LC, GC and GCxGC, and NMR. As the technologies become increasing available, the need for an open exchange of information is required. At The University of Manchester, the HUSERMET (The Human Serum Metabolome in Health and Disease) project[1], funded by the BBSRC and the MRC in collaboration with AZ and GSK, aims to optimise and exploit these technologies in order to develop novel methods for analysis of the human serum metabolome, including appropriate metadata and strategies for data visualisation and modelling. Collections of mass spectra, which includes commonly observed metabolites of either previously identified or unknown chemical structure, exemplifying the most effective ways to pool the identification efforts presently available. The HUSERMET database model presented here is an integrated platform for browsing, querying, annotating and exporting the mass spectral data with their relevant instrumental details (e.g. instrument settings) and clinical information (e.g. patients' metadata). The database model is constructed based on the MeMo model[2], for storing instrumental and experimental data, while additional metadata models were designed for handling clinical metadata collected within the project. HUSERMET model is demonstrated here using omixed[3], a server/client system that permits querying of its databases via web services.

[1] http://husermet.org
[2] Spasic, I., Dunn, W., Velarde, G., Tseng, A., Jenkins, H., Hardy, N.W., Oliver, S.G. & Kell,

D.B. (2006) MeMo: a hybrid SQL/XML approach to metabolomic data management for functional genomics. BMC Bioinformatics, 7, 281.
[3] http://omixed.org

P053 Session 2, Thursday 4:30 - 5:30 p.m.

MetWare: Data Warehousing for Metabolomics

Egon Willighagen (1), Steffen Neumann (2), Mark Fiers (1), Joost de Groot (1), n van Haarst (1)

(1) Plant Research International, Wageningen University and Research Centre (2) Leibniz Institute of Plant Biochemistry, Department of Stress and Developmental Biology

The MetWare [1] project develops data warehouse software to support metabolomics (raw) data and metadata storage as well as data proprocessing and further analysis, such as the detection of biomarkers, etc. A primary design goal is the use of open standards to interact with other tools involved in the aforementioned steps in metabolomics. For example, BioMart and BioMoby webservices are used to provide access to the data, for access in statistics software like R and and workflow software like Taverna, allowing integration with data preprocessing and postprocessing tools available for metabolomics. The design of the warehouse is based on existing reporting standards such as ArMet, and defined in the Web Ontology Language (OWL), including the mapping to table designs for the underlying relational database to allow automatic code generation. To interact with human users a Java Server Faces (JSF) based web front end is developed that allows rich visualization of data in the warehouse, such as mass and NMR spectra, spectral trees, and chemical structures. MetWare [1] is an open source project developed by an international development team consisting of metabolomics groups in The Netherlands and Germany.

1. http://www.metware.org/

P054 Session 1, Thursday 1:00 - 2:30 p.m.

A Metabolomics Datawarehouse

Elwin Verheij (1), Frans van der Kloet (1), Ivana Bobeldijk (1), Cor Kistemaker (1)

(1) TNO Quality of Life

Metabolomics involves the acquisition, handling, storage, and analysis of huge amounts of data from various sources. It is not uncommon in metabolomics to perform the various tasks of the complete workflow with a range of software tools, e.g. LIMS, instrument software, 3rd party processing tools, spreadsheets, databases, Matlab, R, home brew tools and methods, etc. Such workflows tend to be very complex and documentation of actual methods and procedures is often incomplete. Furthermore, data transfer between the various software tools may involve manual, undocumented, handling of data which often leads to errors in the data. This paper presents the metabolomics datawarehousing (DWH) solution implemented at TNO. It is based on SAS Enterprise Guide (4.1) and tailor made tools, providing a single standardized and highly automated data pipeline. The TNO metabolomics DWH consists of integrated functional modules:

- study definition: design, research questions, sample informations
- automated import of measurement data from Thermo LC Quan and Agilent Chemstation
- quality control using predefined analytical performance metrics

- outlier detection/investigation
- scaling, normalisation and calibration of data
- basic statistical analysis tools
- dataset generation for advanced statistical analysis in Matlab

Data and result are made available to users through automated reporting scripts. In addition to the standard reports the end users have access to the data, procedures and results through a secure web portal.

The major advantages of the DWH are:
- central, unified, storage of data
- automated processing, no manual data handling steps
- results can easily be reproduced
- guides execution of metabolomics studies
- strong reduction of data errors, improved data quality
- excellent documentation of study design, raw data, procedures, results, etc.
- automated report generation
- across study data analysis
- platform performance monitoring tool
- time between raw data and data analysis is much shorter

P055 Session 1, Thursday 1:00 - 2:30 p.m.

Datawarehousing: metabolomics workflow optimization

Elwin Verheij (1), Frans van der Kloet (1), Ivana Bobeldijk (1), Cor Kistemaker (1)

(1) TNO Quality of Life

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- Outlier detection/investigation
- Scaling, normalisation and calibration of data
- Basic statistical analysis tools
- Dataset generation for advanced statistical analysis in Matlab
- Web portal

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- data is available after each processing step
- results can easily be reproduced
- guides execution of metabolomics studies
- strong reduction of data errors, improved data quality
- excellent documentation of study design, raw data, procedures, results, etc.
- automated report generation
- across study data analysis
- platform performance monitoring tool
- time between raw data and data analysis is much shorter
- central, unified, storage of data

P056 Session 2, Thursday 4:30 - 5:30 p.m.

Prediction of enzyme-catalyzed reactions based on atomic and molecular properties of metabolites

Fangping Mu (1), Robert Williams (1) , Pat Unkefer (1) , Clifford Unkefer (1) , William Hlavacek (1)

(1) Los Alamos National Laboratory

Metabolomics, the study of a cell's complete complement of small-molecule metabolites, has recently been recognized as an important part of post-genomics science (1). Identification of the structures of metabolites and reconstruction of the network of enzyme-catalyzed reactions in which metabolites participate are two of the important challenges for metabolomic analysis of a cell. Large-scale metabolic network reconstructions currently available have largely been inferred from genome annotation (2), which identifies genes encoding enzymes, and literature data. The sequence-based approach to network reconstruction can only infer reactions that have been characterized experimentally in some system, but large gaps in our knowledge of metabolism seem likely. Unknown metabolic reactions cannot be discovered using automatic reconstruction methods. The gaps in our knowledge of metabolomics are being revealed by metabolomic detection of small-molecules not previously known to exist in cells. An important challenge is to determine the reactions in which these compounds participate, which can lead to the identification of gene products responsible for novel metabolic pathways. To address this challenge, we investigate how machine learning can be used to predict potential substrates and products of enzymecatalyzed reactions (3). Based on 6577 reactions downloaded in the KEGG database (4), we classify the vast majority of these reactions into 80 groups, each of which is marked by a characteristic functional group biotransformation. The functional group patterns of the reaction centers of the 82 substrate(s) types and 88 product(s) types of reactions are represented using SMARTs. These patterns are not unique to these reactions but are widely distributed among KEGG metabolites. Based on atomic properties of atoms in the reaction center, which models the local environment of the functional group, and molecular properties, classifiers have been trained to discriminate the functional groups to be reactive and non-reactive. On average, 80% accuracy is obtained with respect to both sensitivity and specificity. Our results suggest that metabolic reactivity in enzymecatalyzed reactions can be predicted with reasonable accuracy based on functional groups and its local environmental in the molecule, which is characterized by the atomic properties and molecular properties.

1. Fiehn, O. (2002) Metabolomics - the link between genotypes and phenotypes. *Plant Mol Biol*, 48, 155-171.

2. Price, N.D., Reed, J.L. and Palsson, B.Ø. (2004) Genome-scale Models of Microbial Cells: Evaluating the consequences of constraints *Nature Reviews Microbiology*, 2, 886-897.
3. Mu, F., Unkefer, P.J., Unkefer, C.J. and Hlavacek, W.S. (2006) Prediction of oxidoreductase-catalyzed reactions based on atomic properties of metabolites. *Bioinformatics*, 22, 3082-3088.
4. Goto, S., Okuno, Y., Hattori, M., Nishioka, T. and Kanehisa, M. (2002) LIGAND: database of chemical compounds and reactions in biological pathways. *Nucleic Acids Res*, 30, 402-404.

P057 Session 3, Friday 4:00 - 5:30 p.m.

Transformation function to improve biological interpretation of metabolomic data

Gerhard Koekemoer (1), Carools Reinecke (1)

(1) North-West University

The multivariate data generated in metabolomic experiments produce metabolites (variables) for which the scales are mostly not comparable, including a large number of metabolites consisting of zero values. These two characteristics of the data can complicate the multivariate statistical analysis. In addition, these differences may not be proportional to the biological relevance of the metabolites. However, methods of data analysis are not able to make this distinction, which necessitates that some data pretreatment methods should be introduced. It has thus been shown that proper data pretreatment is an essential step in the analysis of metabolomics data and can greatly affect the information encapsulated in the data set[1]. As a primary interest in a metabolomics experiment is to compare a control situation with a given perturbation (e.g. environmental influences, inherited or acquired disorder or genotypic variation), it is important to separate *interesting* biological variation from possible *obscuring* sources of variability intrinsic in the data. This poster presents some results which address these issues.

Normally, the zero values in the data are replaced by half of the detection limit, after which standardization or a transformation function is applied to the data; with the aim to stabilize the variances of the different variables (hence force variables with high variation not to dominate the statistical analysis). In this poster we introduce a *new semi-parametric transformation* function which is more flexible in assuming different shapes (i.e., concave, convex, concave to convex, convex to concave, and any combination of the above), easy to apply, and can be iterated. For the application of this transformation function, distribution estimation is required. Hence, the zero values need to be handled differently. For this purpose, we propose the use of a compactly supported probability distribution function which includes a large number of possible distributions. The choice of parameter selection for the replacement of zero values and the effect of this choice on multivariate analysis are presented. The proposed procedure is compared to alternative procedures such as auto-scaling, range-scaling, and pareto-scaling.

We used a data set consisting of 475 variables (metabolites expressed as mg/gr creatinine) obtained from the analysis or the urinary organic acids in 19 controls and 18 patients suffering from propionic asidaemia. Propionic acidaemia (PA) is a life-threatening inborn error of metabolism with autosomal recessive inheritance, caused by deficiency of propionyl CoA carboxylase (PCC, EC 6.4.1.3). The organic acids were isolated according to standard procedures, derivatized and analyzed on a GC-MS. The proposed procedures as well as additional statistical analysis are presented for this dataset.

Conclusions:

1. We claim that the semi-parametric transformation function transforms the raw data from a metabolomic experiment to clean data, which is statistically more acceptable.

2. Since the proposed transformation function is unique to the dataset at hand and is fully automatic, it makes application and further statistical analysis easier.

(1) Van den Berg, RA, Hoefsloot, HCJ, Westerhuis, J., Smilde, AK and van der Werf, MJ, BMC Centering, scaling and transformation: improving the biological information content of metabolomics data, Genomics, 7**,** 142 – 157, 2006

P058 Session 1, Thursday 1:00 - 2:30 p.m.

BinBase, a GCTOF mass spectral database for high quality processing of dissimilar chromatograms

Gert Wohlgemuth (1), Oliver Fiehn (1)

(1) UC Davis Genome Center

Chromatography-coupled mass spectrometry yields hundreds to thousands of metabolic signals per sample. Existing solutions for peak picking and data alignment rely on similarity matching across all samples of an experimental batch. This approach fails if highly dissimilar chromatograms are to be compared, e.g. urine and plasma, in order to find metabolites that are shared in both organs or to compare signals that would be specific for one matrix but not found in the other. In addition, existing solutions often rely on fixed spectra libraries and assign compound identifications without sufficient quality criteria thresholds.

We have therefore constructed an algorithm using a multiplexed threshold filter system that builds upon the peak picking and mass spectral deconvolution capabilities of Leco's ChromaTOF software. The processing algorithm relies on retention index markers (C8-C30 fatty acid methyl esters) according to long established practice in GC/MS, instead of relying on retention times alone which would shift due to column aging or sample overloading. The retention index system is most important to compare dissimilar chromatograms as it aligns peaks on a fixed grid of marker compounds without relying on overall sample similarity. Furthermore, the filter system (using further mass spectral metadata such as s/n, apex ions, unique ions and peak purity) enables the automatic addition of novel metabolites into the BinBase database without manual interaction. This feature allows discovery of metabolites that are unique to certain cell types, organs or diseases in a coherent manner. Eventually, novel biomarkers and reports are based on the consistent detection in a group of representatives samples (a 'class' defined in our SetupX study design database), for which we usually employ a 80% positive detection threshold.

As result, around half of the detected peaks in a chromatogram are discarded as signals are too noisy, too low abundant or not consistently detected in a given matrix. This feature helps subsequent statistical studies as the overall data set contains less noise and because artifact compounds (such as column bleed polysiloxanes) are not reported. Currently, BinBase holds over 2400 compounds which are matched against over 1,100 spectra recorded in the Fiehn GC/MS libraries and further spectral resources such as the NIST05 library. BinBase reports unknown metabolites by retention index, full mass spectra, quantification ions and unique database identifiers, whereas chemically identified compounds are further annotated by names, KEGG and PubChem entry numbers.

BinBase is programmed in Java on open source APIs and runs on a rocks linux cluster. Documentation and source codes are downloadable under the LGPL license. It currently holds data for almost 11,000 samples of 26 species with around 30 million mass spectra. Example studies can be downloaded including raw data (with netCDF formats), devonvoluted peak lists (*.csv) and final processed data (XML and *.xls)

P059 Session 2, Thursday 4:30 - 5:30 p.m.

MassBank : A Mass Spectral Database for Metabolomics Introduction Mass

Hisayuki Horai (1) , Masanori Arita (2), Shigehiko Kanaya (3), Takaaki Nishioka (1)

(1) Keio University (2) University of Tokyo (3) Nara Institute of Science and Technology

Spectra of metabolites are analyzed on different types of mass analyzers with various ionization methods. This makes difficult to share and exchange mass spectral data. In 2006, MassBank started to collect mass spectra of metabolites. MassBank defines a standard record format to describe a set of detail information on mass spectral experiments that is along the guidelines and standards for sharing and reporting experimental data proposed by Metabolomics Society. We report the current status of MassBank. MassBank Web Service MassBank is a distributed database in the internet so that many researchers can contribute their spectra on their own servers easily. It is expected to enlarge the number of participants and spectra and to collect mass spectra of metabolites comprehensively. MassBank provides high speed spectral search web services based on a vector space search algorithm and expanded for real-valued *m/z*. To compare a query and searched spectra easily, we introduced a view which can align them graphically in three dimensions. It also provides a peak search by difference between a pair of *m/z* values related to a common substructure of metabolites and a batch service which accepts a file containing more than two query mass spectra and send all results to the user by e-mail. Spectral Data The database will cover the comprehensive compilation of diverse spectra from biological samples, for instance, natural extracts and standard metabolites in libraries. Currently, MassBank provides 13,563 spectra of 1,723 compounds including 106 carotenoids and 42 lipids. The spectra are measured in several MS methodologies including QqTOF-MS/MS, IT-(MS)n, LC/QqTOF-MS/MS, QqIT-MS/MS, GC/TOF-MS, CE/TOF-MS and FAB-MS. Five laboratories contribute their spectra currently and 4 others are preparing to contribute their data. The servers are installed not only for the public services but also for their internal uses. An annotated text database record is provided for each spectrum. It includes equipment information, experimental conditions, peak identification and material information such as synonyms, SMILES code, InChI code and links to KEGG, CAS, and PubChem databases. Each record can be browsed on the web service with the graphical spectrum and the chemical structure.

P060 Session 3, Friday 4:00 - 5:30 p.m.

Model Selection using L1-Norm in Metabolomics

George Dougherty (1), Jeffrey Yao (1)

(1) VA Pittsburgh Healthcare System

Metabolomic models potentially involve large numbers of parameters. Examples are the coefficients of a discriminant function used to separate groups of samples, or a correlation matrix among metabolites in a pathway. All these parameter estimates contain error. Many may be statistically not different from 0. In order to estimate the (nonzero) parameters by Maximum Likelihood, one needs to decide in advance which ones are 0, a decision called `model selection.' Model selection usually proceeds by choosing an estimate of model prediction error (e.g., average proportion of misclassifications for a discriminant function applied to new data), which the `best' model will minimize. Then, often, one either adds parameters to the model starting with one (forward selection, FS), or deletes parameters from the model starting with all (backward elimination, BE), or both in alternation (FS/BE). At each step, many models are estimated and checked, as one runs through all candidates for the next parameter to be added or dropped. The approach is

computationally intensive, and still misses most of all possible models. *L1-Norm methods* modify the likelihood function (L) so that maximizing it, even with all the parameters included, will produce parameter estimates with some assigned to 0. The modification is a `penalty' term which can have its own multiplier to control how many 0's there are among the parameter estimates. Hence, FS/BE is replaced by a single sequence of optimizations. Fast algorithms are now available for some L1 problems. Recently, the *L1-Norm method* has been applied to *"Gaussian Graphical Model" selection*. In our work with correlations of pathway analytes, this amounts to finding which conditional correlations are statistically 0 in a `best' model. The final matrix choice is displayed conveniently as a "graphical model" with analytes as "nodes" connected by "edges" that represent nonzero conditional correlations. The method is illustrated using amino acid concentrations measured in the CSF of normal control subjects and patients with schizophrenia. The L1 approach appears to achieve reasonable parsimony with better speed than FS/BE. (Supported by VA Merit Review Grant).

P061 Session 1, Thursday 1:00 - 2:30 p.m.

Bayesian curve fitting using Gaussian Process for longitudinal metabolomics data

Jing Tang (1), Marko Sysi-Aho (1), Tuulikki Seppänen-Laakso (1), Olli Simell (2), Matej Oreši (1)

(1) VTT Technical Research Centre of Finland (2) University of Turku

Metabolomics datasets in time series experiments or longitudinal cohort studies consist of measured metabolite concentrations across multiple samples over time. Many studies attempt to find subsets of metabolites that exhibit different temporal profiles between samples corresponding to different subject groups, e.g., disease cases and controls. Traditional Least squares regression provides a point estimate of the best curve-fitting by igoring the confidence intervals, and thus is sensitive to the noise level in the data.

Here we show that Gaussian Processes (Rasmussen and Williams, 2005) can provide a flexible statistical framework for modelling of time-series metabolomics data. Unlike the Lease square regression, Gaussian processes model explicitly the data generation and measures the posterior uncertainty of the curve fitting via Bayesian rules. We use data from a large type 1 diabetes birth cohort data for illustration of the method. To reduce the noise level, we preprocess the data by binning and then apply the Gaussian processes with maximal likelihood estimation. The results show that a subset of lipids in which the temporal expression patterns between groups, such as case/control and male/female, can be diffentiated with well-defined confidence intervals.

P062 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolomics Information and Tools Available from the BMRB and MMCD

John L. Markley (1), Qiu Cui, M. Francisca Jofre (1), Ian A. Lewis (1), Christopher F. Schulte (1), Eldon L. Ulrich (1)

University of Wisconsin-Madison

BMRB (BioMagResBank) has developed an archive of NMR data for metabolites and other small molecules found in biological systems located at: http://bmrb.wisc.edu/metabolomics. The website provides primary data (time-domain spectra) as well as processed spectra and assigned chemical shifts. The site has been populated with data collected by the Madison Metabolomics Consortium

(MMC) and more recently by the Human Metabolome Database (deposition ongoing). The MMC deposition includes 1D ^{1}H, 1D ^{13}C, DEPT90, DEPT135, 2D [^{1}H,^{1}H]-TOCSY, [^{1}H,^{13}C]-HSQC, and 2D [^{1}H,^{13}C]-HMBC time domain data sets collected under defined conditions, processed spectra, and assignments from a growing number (currently ~477) of pure compounds from a collection of 986. A deposition tool under construction at BMRB will enable others to submit data to add to this collection. The BMRB site is mirrored by PDBj in Osaka and CERM in Florence, Italy. The site contains versatile search tools. The MMCD (Madison Metabolomics Consortium Database), located at http://mmcd.nmrfam.wisc.edu collects additional information on more than 20,000 compounds (metabolites, xenobiotics, and environmental chemicals). Each compound entry in MMCD is accompanied by a comprehensive synonyms list, chemical formula, structure, physical and chemical properties, predicted NMR chemical shifts, experimental NMR spectra (if available), average and monoisotopic masses, MS/MS data (if available), information on related metabolic pathways, occurrence in various biological species and direct links relevant records in other public databases. A sophisticated search engine enables complex searches that can incorporate text, molecular structure, NMR chemical shifts, LC-MS or MS/MS data, and or other parameters. Newer tools available from the MMCD include *easyNMR* (a XWIN-NMR extension for automatic NMR data collecting and processing), and *easyMETA* (software for automating the analysis of NMR spectra of mixtures of compounds, such as compound identification, quantification, and metabolite profiling).

Supported by NIH grants R21 DK070297, P41 RR02301, NHGRI 1T32HG002760 (fellowship for I.A.L.), and P41 LM05799 and by DOE support for the Great Lakes Bioenergy Consortium.

P063 Session 2, Thursday 4:30 - 5:30 p.m.

Visualisation, Alignment and Data Mining on MCC/IMS-Signals

Jörg Ingo Baumbach (1), Alexander Bunkowski (1), Bertram Bödeker (1), Sabine Bader (1), Sandra Volker (2)

(1) ISAS - Institute for Analytical Sciences, Department of Metabolomics (2) Statistics Department at the Technical University Dortmund

The effective and fast data processing in ion mobility spectrometry is a rather unsolved problem, especially with respect to couplings to fas gas-chromatographic columns. Over the last years unflexible, mostly firmware dominates the field of software solutions for applicants. Recently, the development of more open and scientific solutions are developed, especially for use in metabolic profiling and for clinical applications, far from simple yes/no answers occuring for the detection of explosives or chemical warfare agents. To provide validated solutions for visualisation, alignment, peak finding, peak interpretation, cluster analysis and data mining the cooperation of natural scientists, engineers, information scientists and statisticians is needed.

A software tool developed at ISAS – Institute for Analytical Sciences in cooperation with the Technical University Dortmund and the University Bielefeld will be described in detail. A software platform for visualisation, pre-paring of data sets, alignment and data mining will be described. Proven applications will be shown with respect to profiling of metabolites in human breath and on different cancer cell lines.

P064 Session 1, Thursday 1:00 - 2:30 p.m.

Global Metabolite Biomarker Discovery Using Novel Visualization of GCxGC-TOFMS Data

Lingyan Liu (1) , Wei Chen (2) , David S. Ebert (3) , Daniel Raftery (4)

(1) Biomedical Engineering, Purdue University (2) State Key Lab of CAD&CG, Zhejiang University (3) School of Electrical and Computer Engineering, Purdue University (4) Department of Chemistry, Purdue University

Two-dimensional gas chromatography coupled time-of-flight mass spectrometry (2DGC-MS) was first introduced in the 1990s and has gained interest steadily because of its powerful resolution and identification capabilities. Recently, 2DGC-MS has been used in metabolomics studies for early disease detection and for systems biology research. However, since metabolomics samples typically produce data intensive, complicated and high dimensional GCxGC-TOFMS spectra, data handling and spectral analysis create significant bottlenecks in the analysis pipline, especially for untargeted biomarker discovery efforts. In this study, we explore new visualization methods to improve the global detection of biomarkers. Our in-house developed software allows fast comparisons of control and disease samples and couples them with individual or averaged mass spectra. Interesting potential markers can be easily identified using chosen thresholds of selective filters such as loadings, p-values, and ROC areas. Subsequently, specific mass values can be used to quantify each compound. The total pipeline empowers quick differentiation of potential bio-markers in a visual format that uses the mass spectra. We applied this method on a set of metabolomics sample set, comprising 102 samples in total, 53 and 49 of disease and healthy samples respectively. The efficiency at each step improves from 2 to 16 fold.

P065 Session 2, Thursday 4:30 - 5:30 p.m.

FlaGMe: Fragment-level analysis of GCMS-based metabolomics data

Mark Robinson (1), Ian Gentle (2) , David De Souza (3), Martin O'Hely (1) , Eleanor Saunders (3)

(1) Walter and Eliza Hall Institute of Medical Research (2) La Trobe University (3) University of Melbourne

Gas chromatography in combination with mass spectrometry (GCMS) is a widely utilized platform for untargeted metabolomics studies. Data processing can be a major hurdle, requiring several steps such as peak detection, alignment, and normalization before any downstream statistical analysis. We propose a new suite of methods that cover the full pipeline of data processing, including a dynamic programming strategy for alignment and the downstream statistical analysis, all in a single computing environment. The approach is modular and can be used in conjunction with various instruments, peak detection algorithms (e.g. AMDIS) and alternative alignment procedures.

The main novelty of our approach is the full use of observed fragment information. Where possible, the intensity of each metabolite fragment is collected and used in the downstream analysis. This proves beneficial for: i) determining changes in metabolite abundance between experimental conditions for profiling experiments, and ii) estimating ^{13}C enrichment in heavy-isotope tracer experiments. We discuss some further enhancements over standard methods, such as explicitly dealing with missing data and using statistical methods that share information across all observations for improved inferences.

We illustrate the proposed method on two benchmarking datasets and one biological experiment, showing it can outperform standard tools. The software was developed as an add-on package to R (http://www.r-project.org), a freely available and rich environment for data analysis and visualization.

P066 Session 3, Friday 4:00 - 5:30 p.m.

JDAMP: A Software Tool for Differential Analysis of CE-MS based Metabolome Data

Masahiro Sugimoto (1), Akiyoshi Hirayama (1), Takamasa Ishikawa (2), Richard Baran(3), Martin Robert (1)

(1) Keio University (20 Human Metabolome Technologies Inc (3) Genoscope-Centre National de Séquençage

Rapid identification of quantitative differences in multiple biological samples is a major challenge in metabolomics. Capillary electrophoresis-mass spectrometry (CE-MS) is a versatile analytical platform well-suited for metabolome studies where efficient compound separation and high sensitivity are required. CE enables temporal separation of metabolites based on their charge and size, while MS provides efficient differentiation for those compounds that co-migrate in CE. The main drawbacks in CE-MS include widely non-linear migration time shifts between samples and a wider diversity of peak shapes and sizes compared to LC-MS or GC-MS. To solve these problems and simplify CE-MS data processing, we implemented a stand-alone Java application named JDAMP (Java application for Differential Analysis of Metabolite Profiles) that works on freely available libraries. The software executes a typical analytical flow starting from data import and for this we developed high-throughput file converter that imports CSV or Agilent .wiff file and exports original binary format file. The following steps include binning, background subtraction and denoising, migration time-shift correction, signal intensity normalization, and detection of significant differences between multiple samples. The migration time shift correction based on dynamic programming with simplex optimization and the difference detection without peak picking were designed specifically for robust and sensitive of CE-MS based data. The simple graphical user interface (GUI) enables users to execute all procedures automatically using initially selected parameters, or run each process step-by-step, for better quality control, by confirming each result and/or interactively re-doing any process using different parameters, as necessary. The GUI also visually highlights the location of metabolite differences and known compounds on 2D plots (m/z and time axis) and generates analysis reports in CSV or HTML format. Immediate confirmation of findings is available as a list of overlaid electropherograms ranked by statistical significance. For large-scale analyses, a calculation engine was implemented in C++. Overall, the JDAMP software allows easily and rapidly detecting significant differences in multiple complex CE-MS profiles. The software and documentation are freely available at http://software.iab.keio.ac.jp/jdamp for non-profit research purposes

P067 Session 1, Thursday 1:00 - 2:30 p.m.

SetupX – a study design database compliant to the Metabolomics Standards Initiative (MSI)

Martin Scholz (1), Oliver Fiehn (1)

(1) UC Davis Genome Center

We present a solution for setting up metabolomic experiments that is compliant to minimal reporting standards as suggested by the Metabolomics Standards Initiative (MSI).

SetupX comprises the description of biological study designs, the management of the experimental lifecycle and it also serves as a public repository and download interface for metabolomic studies. Metabolomic data cannot be interpreted without accurate description of the underlying metadata that detail the experimental setup, ranging from the organisms, organs and cells that are studied to the genotypes, treatments and time courses that are employed. SetupX further demands to detail laboratory protocols from sample preparation to data processing in order to be provide consistent experimental details and enable researchers to understand and re-use data that eventually result from the metabolomic studies. SetupX therefore aids researchers to carefully design and completely document their studies with the added benefit that studies are laid out in a systematic manner and can be queried on different levels (e.g. species, treatment or other search terms). Current approaches in metabolomic databases do not support such details in machine-readable and systematic formats, rendering SetupX a novelty in the field. Ensuring data integrity of these designs is accomplished by utilizing publicly available taxonomic and ontology repositories and the standards of the Metabolomics Society (MSI). The system currently hosts studies on various species (6,372 plant samples, 1,103 microorganism, 2,337 animal and 711 human). A subset of 1,614 out of the total 11,102 samples is publicly available, including the complete experimental design, raw and annotated GC-TOF result data. Additional samples and studies become publicly available as laboratory collaborators publish their findings.

Secondary to its function as experimental metadata repository, SetupX acts as a LIMS. It combines all files relevant to a study in a central place, enabling the operator to submit result data from different technologies (GCMS, LCMS or direct infusion FTMS) in a single environment. Scheduling and randomizing samples is directly connected to the Leco GCTOF mass spectrometer to aid laboratory assistants. SetupX further enables the lab managers and administrators to gain overview of scheduled experiments, current data acquisition and status of data processing in real time. SetupX is seamlessly integrated to the BinBase GCTOF database, which uses the study design for statistical purposes.

SetupX therefore presents a fully functional and public database system integrating metabolomic workflows from conceptual design over laboratory practice to steering data processing tasks and result queries. SetupX is an open source project and can be downloaded and installed under the LGPL license.

P068 Session 2, Thursday 4:30 - 5:30 p.m.

WIKI-BASED DATABASE WITH EMBEDDABLE QUERIES

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(1) The University of Tokyo (2) Kyoto University (3) Nara Institute of Science and Technology

Database systems in computer science are based on the relational model, whereas in biology areas the word ‘database’ often refers to loosely formatted, very large text files. Although such bio-databases may describe conflicts or ambiguity in a positive sense, the flexibility in the data format sacrifices a systematic query mechanism equivalent to the widely used SQL. By implementing embeddable string-search commands on a Wiki-based system, we have designed a database system that takes advantage of both database models: registered users can edit information in an arbitrary format while structured texts are subjected to page searches to realize relational-database operations. As a proof of principle, our half-formatted database (accessible at http://157.82.238.146/wiki/) provides 6851 flavonoid structures from over 1687 plant species on MediaWiki. The system was written in PHP language as the extension of MediaWiki. All codes are

open-source. The introduction of embedded string-searches raised the notion of (web) page dependency. Dependent pages can be categorized into 3 types, each corresponding to standard Wiki pages, CGI-based pages, and RSS-like pages. This categorization sorts out seemingly irrelevant web functionalities in a streamlined manner, and renders the task of data input and maintenance much easier. For example, automated generation of index pages or statistic pages became possible through the above mentioned mechanism (RSS-like pages and CGI-based pages, respectively). The systematic (*i.e.* relational) queries, gives each user a power to design and construct summary or 'meta' knowledge pages as is done by Google on the net. The system is extendable to all metabolite categories, and data integration of other classes of metabolites, plant source information, and their mass spectra from different institutes is ongoing

P069 Session 3, Friday 4:00 - 5:30 p.m.

Identifying biomarker metabolites in complex biological systems: A statistical approach

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Any biological system is far more complex than that of a physical system of inert matter and a deep level of analysis is needed to deal with such complexity. In this regard, statistical methods could represent the ways to deal with such complexity. The essence of a biological system in normal or environmentally challenged conditions can be putatively defined by a number of proteins/metabolites. Some of these detected proteins/metabolites could be used as biomarkers for further investigations. So far, detecting the biomarkers in biological systems has been a challenge among scientist. To meet this challenge, we designed a high throughput statistical workflow for data analyzing and biomarker identification in complex biological datasets. As a test example we applied this workflow to a metabolome dataset originated from GC-TOF-MS technology, where the underlying biological question was related to plant salt stress physiology. Experiments were performed on two tolerant (Pokkali and IR57311) and three sensitive (Tai Pai, Nipponbare and Zhong Hua) rice cultivars under three salt stress doses (0, 50 and 100 mM NaCl). The results lead us to discover 26 general, 14 tolerant and 41 sensitive marker metabolites in root and/or leaf tissue, each of which could be used for further investigation. Here we present the results and show the ability of this workflow for biomarker discovery in complex biological systems.

P069-2 Session 2, Thursday 4:30 - 5:30 p.m.

A statistical toolkit for metabolomics

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A statistical toolkit has been written for treatment of NMR and MS data in Metabolomics. The toolkit is written in Matlab (www.mathworks.com) and uses the Eigenvector (www.eigenvector.com) DataSet Object for data input but it is self-contained, requiring only the core Matlab installation. Most operations are carried out via a single screen GUI and require no expertise in Matlab itself. It has been designed to be easy for non-statisticians to learn and use but validation procedures have been built in as an integral part of the software to avoid the danger of overfitting.

Several methods are included but the main multivariate application is based on non-orthogonalized PLS1, specifically for handling two-group classification problems. The PLS factorization is split in a

post-processing step into y-relevant and y-unrelated parts (analogues of the "filtered data" and "orthogonal components" in Trygg's O-PLS). The key results from the analysis are contained in a single scores plot and a single loading (for the two-group case) and this greatly simplifies the interpretation of the model compared with other multivariate methods such as PLS-DA. The simplification is particularly valuable in cases where effects of the treatment of interest are quite modest but other major unrelated sources of variance are present. This situation is quite typical e.g. in nutritional intervention studies with human subjects.

The different steps of an analysis will be illustrated with the help of NMR and LC/MS datasets:

1. DATA INPUT: data overview, visualisation and pre-processing
2. INNER (train/tune samples) VALIDATION, followed by OUTER (test samples) VALIDATION done once.
3. INNER (train/tune) and OUTER (test) VALIDATION, with repeat partitioning – OPTIONAL
4. REPORT: summary report in html format, individual figures saved for export to other applications.

Step 2: the data are partitioned into modelling and test sets, either randomly or with the option to specify samples. The modelling set is randomly partitioned into training and tuning segments, PLS1 is carried out on the training set and models of increasing dimensionality, up to a specified maximum, are applied to the tuning set to determine the classification success rate. Classification is made using the single score obtained by projecting the y-related part of the PLS factorization onto the first PLS loading. The procedure is repeated as many times as required with resampling of the modelling set into training and tuning segments. This defines an optimum dimension for the model which is then calculated from the entire modelling set. The model is applied to the test set to give a realistic estimation of the classification performance.

Step 3: this option repeatedly repartitions all of the data into training, tuning and test segments. A model with the optimum dimensionality from step 2 is calculated each time. The mean classification success rate for the test and other sets is reported together with histograms showing the success rate distribution with resampling. The mean of the first PLS loading obtained from the modelling set resamples is calculated, together with the loading signal-to-noise from the resampling.

The entire calculation takes a few minutes, even with 100-fold resampling in both steps 2 and 3. In addition to the summary report, detailed results from each step (score, loading, y-relevant part of the data, etc.) are exported to Matlab structures from where they are readily available.

P070 Session 1, Thursday 1:00 - 2:30 p.m.

EasyNMRMETA: an "easy-button" for metabolomics research

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A metabolomics project normally must handle hundreds of samples. The collection and analysis of these samples can present a bottleneck for successful metabolomics research. To address the need for fast and automated high-throughput analysis, we have developed the easyNMRMETA tools. The easyNMRMETA software package, which is available from the website of the Madison Metabolomics Consortium Database (MMCD, http://mmcd.nmrfam.wisc.edu), can automate a typical NMR-based metabolomics study from data collection, through data processing, metabolite identification and quantification, and PCA analysis. EasyNMRMETA consists of two components: easyNMR and easyMETA. The easyNMR component is a Bruker XWIN-NMR extension designed to perform automatic data collection and data processing. The easyNMR software automatically sets up

common 1D and 2D NMR experiments used in the metabolomics field, such as 1D-^{1}H, 1D-^{13}C, 2D-HSQC/HMQC, 2D-HMBC, and 2D-[^{1}H, ^{1}H]-TOCSY. The flexible design of easyNMR makes it easy to add new customized NMR experiments to the system. After the sample is loaded into the NMR spectrometer, the user chooses which experiment(s) to run, and easyNMR automatically directs a series of chosen NMR operations, such as lock, shimming, pulse width calibration, experiment set up, data acquisition, FT, Phase collection, and chemical shift referencing. The easyMETA component is a pure Java-based client that uses Java Web Start technology. EasyMETA uses the MMCD as a backend and automatically updates the software itself and the available data content from the MMCD. EasyMETA is designed mainly for the identification and quantification of compounds in mixtures on the basis of a 2D NMR spectrum, such as ^{1}H-^{13}C HSQC. The easyMETA package accepts data generated by easyNMR or spectral data in a variety of formats: those currently supported include Bruker format (.2rr), Varian format, NMRPipe format(.ft2), Sparky format(.ucsf), and Felix format(.mat). The functionality that easyMETA provides includes: visualization of 1D/2D spectra, automatic peak-picking and noise filtering, compound identification and quantification, peak assignment, adaptive reconstruction of spectra of mixtures from spectra of standards in the database, visualization and comparison of standard spectra overlaid on the experimental input spectrum, and PCA/metabolite profiling. EasyMETA operates under Windows and Linux platforms.

Supported by NIH grant P41 RR02301.

P071 Session 2, Thursday 4:30 - 5:30 p.m.

TNO-DECO, a comprehensive tool for processing of GC-MS data

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Analytical chemistry by means of chromatography and mass spectrometry is used in many types of research where the composition of body fluids or body tissues needs to be known. Chromatography is used to separate the different compounds within a mixture and mass spectrometry (MS) is used to detect details about the identity and concentration of the compounds present. In complex matrices that are common in metabolomics, it becomes impossible to separate all compounds in a practical runtime. As a result, peaks will overlap and standard procedures for peak integration are not feasible. Still, a list of compound concentrations and corresponding mass spectra identifiers are required.

Therefore, we tackle the problem of peak overlap by adding the mathematical technique of deconvolution to the chemical and spectral separation techniques of chromatography and mass spectrometry. Using the approach of deconvolution assures: (1) utilizing the information enclosed in the data derived with chromatography techniques common for metabolomics and (2) deriving this information in a reasonable amount of time.

This statement holds in case of a so-called non targeted approach in which all measured peaks are extracted automatically from a chromatographic profile. In contrast, targeted approaches exist that are manual and are mainly based on experts' opinions but are much more time consuming and can be subjective. While fast instruments are becoming more commonly used, the processing of the measured data must be enhanced to keep up with the pace of the instrumental developments.

Deconvolution as such is not new and freely available tools such as AMDIS can be downloaded for use by analytical experts. Unfortunately, the currently available tools are not suitable yet for

automatic processing of chromatography mass spectral data especially in the field of metabolomics where non targeted approaches are common. In this paper we will present the current status of TNO-DECO, a comprehensive tool that allows for automatic deconvolution of multiple data files including visualization and fault detection.

P072 Session 3, Friday 4:00 - 5:30 p.m.

Principal Component Variable Grouping (PCVG); A tool to reduce the data "load"

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Principal component analysis (PCA) is a powerful tool for exploratory data analysis that reduces data dimensionality and visualizes the sample and variable behaviour. PCA finds combinations of variables (principal components, PCs) that explain the variance in the data, and the results are scores that reflect the behaviour of the samples and loadings that correspond to combinations of variables. It is often straight forward to understand scores plots since there are often fewer samples than variables, especially for techniques such as LCMS that can generate large quantities of data, but interpreting the loadings and the meaning of the variable combinations can be much more difficult.

Principal component variable grouping (PCVG)1 is a tool that analyzes the loadings plots in an intuitive way to find groups of correlated variables. Because the groups use the original data space, they are easier to interpret than the PCs themselves. The members of a group have the same behaviour across all samples, which can be displayed as a profile plot and examined for interesting or uninteresting effects, or artifacts2. Group members may arise from the same or different compounds which can be clarified using retention time information. The entire group, or individual compounds, can be represented by single variables that can be used in further processing. We describe PCVG and some of its applications.

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P073 Session 1, Thursday 1:00 - 2:30 p.m.

Defining the Unique Biomarkers of Cancer Cachexia with Metabolomics

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Cancer cachexia is best defined as uncontrollable and unintentional weight loss due to skeletal muscle and adipose tissue wasting. It is a devastating and understudied illness in patients with gastrointestinal, pancreatic, lung, and head and neck cancer. It results in weakness and immobility, and is associated a marked reduction in the quality of life. The presence of cancer-related cachexia often complicates and limits the treatment options for patients. In advanced cancers, greater than 50% of patients exhibit cachectic symptoms and greater than 20% of all cancer patients will succumb to complications related to cachexia. The diagnosis of cachexia remains the subject of significant controversy. Current methods of detection rely on clinical assessment and crude measures of inflammation such as C-reactive protein (CRP). Accurate diagnosis is essential since merely

increasing nutritional intake is not enough to treat cancer cachexia. Although it is known to be distinctly different from starvation, there is still much to learn about the metabolic alterations that arise from cancer cachexia and how to best detect and monitor the therapeutic responses.

Many cancers remain localized and do not result in systemic perturbations to the metabolome, but the systemic nature of cachexia makes well suited for study by metabolomics. In this study we have applied global NMR-based metabolomics to a set of murine models to determine the unique set of biomarkers that distinguish cancer cachexia from starvation and the effects of a large tumor burden. We recently reported that the metabolic profile of cancer cachexia in a murine model is clearly distinguishable from controls. This study involved inoculating mice with a C-26 adencarcinoma cell line which grew into a solid tumor. Serum was collected at three time-points (1) prior to inoculation (2) after palpable tumors were found, but prior to significant weight loss and (3) after the development of cachexia as determined by muscle wasting and weight loss. The cachectic mice were characterized by prominent alterations in lipid and carbohydrate concentrations (O'Connell, *et al*, Metabolomics, 2008 in press). To determine the distinctions between cachexia and starvation, a metabolomic analysis was carried out on a set of mice with no tumors and a diet with approximately 40% reduction in caloric intake as compared to controls. The serum metabolome of the calorie restricted animals displayed distinct elevations in ketone bodies such as 3-hydroxybutyrate and acetoacetate. The metabolic effects of a large tumor burden were studied by inoculating mice with P388 lymphoma cells which resulted in a non-cachexia inducing solid tumor. The metabolome of these mice were characterized by perturbations in several metabolites including decreases in serum glucose and carnitine levels. Taken together these models show that cancer cachexia is metabolically distinct from starvation or the effects of a large tumor burden. This first application of metabolomics to cancer cachexia holds significant promise toward the development of a metabolomic diagnostic tool for cachexia.

P074 Session 2, Thursday 4:30 - 5:30 p.m.

A comparison of classification methods for metabolomics data analysis

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The analysis of most large scale '- omics' data generally involves one of two approaches that reduce the dimensionality of the data by projecting the original variables onto a lower dimensional "latent variable" space. The approaches are: (a) linear classification techniques such as principal component analysis (PCA) and partial least squares discriminant analysis (PLS-DA) and, (b) nonlinear classification methods such as artificial neural networks (ANN) and support vector machines (SVM) among others. In metabolomics applications, linear classification techniques especially PCA and PLS-DA predominate. However, over the recent past, arguments have arisen that some linear classification methods yield results that are either false or hard to interpret.

In this work we use several experimental NMR-based metabolomics data sets to illustrate the performance of common linear classification methods (PCA, PLS-DA and projection pursuit) on their ability to recover inherent biological information from these data, and point out advantages and disadvantages of each method. Using simulated examples we show that depending on the extent and nature of the variance exhibited by the data, some methods yield suboptimal results. For instance, we show that in cases where the between group variance is smaller than the within group variance, PCA is a suboptimal method for classification. Furthermore, depending on the characteristics of the noise in the data, recovery of inherent information will heavily depend on the pre-processing technique employed. To this end, we compare methods for data pre-processing, i.e.,

mean centering, Pareto scaling, auto-scaling and g-log transformation to maximum likelihood principal component analysis (MLPCA), a method for maximum likelihood subspace estimation that has been in the chemometrics literature for over a decade.

P075 Session 3, Friday 4:00 - 5:30 p.m.

Development and application of a differential method for reliable metabolome analysis in Escherichia coli

Hilal Taymaz Nikerel (1), Marjan de Mey (2), Jan van Dam (1), Joseph Heijnen (1), Walter van Gulik (1)

(1) Delft University of Technology (2) Ghent University

Quantitative metabolomics of microbial cultures requires well designed sampling and quenching procedures to obtain proper snapshots of intracellular metabolite levels. Accurate measurement of these intracellular metabolite concentrations requires efficient removal of the surrounding medium if these metabolites are also present outside the cells. LC-ESI-ID-MS/MS and GC-ID-MS analysis of the culture filtrate of steady state chemostat cultivated Escherichia coli K12 cells showed that significant amounts of glycolytic, pentose phosphate (PP) pathway and TCA cycle intermediates, free amino acids and nucleotides were indeed present in the cultivation medium. The most commonly applied method for removal of the surrounding medium is quenching of culture samples in cold methanol and subsequent washing of the cell pellet. From a rigorous analysis of this quenching procedure, whereby metabolite analyses were carried out in total broth, culture filtrate, cell pellets and the obtained supernatants after quenching and washing of the cell pellets, it was concluded that cold methanol quenching was not applicable because it resulted in release of a major part (> 80%) of the metabolites from the cells. No positive effect of buffering (with HEPES of tricine) or increasing of the ionic strength of the quenching solution by addition of NaCl was observed. Therefore a differential method was developed and applied, whereby metabolite measurements in total broth samples were corrected for the metabolites present in the culture filtrate. Different methods for sampling of culture filtrate were examined and it was found that direct filtration without cooling of the sample prior to filtration was the most appropriate procedure. The differential method was successfully applied to obtain a thermodynamically consistent metabolite dataset for chemostat cultivated E. coli cells.

P076 Session 1, Thursday 1:00 - 2:30 p.m.

Towards a unified database for metabolites characterization

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NMRShiftDB is an open submission, open access, open source database of organic molecules, mostly natural products, and their 1D NMR data, instantiated by our group. Most of the data in NMRShift were contributed by the community. Here we present NMRShiftDB and it's underlying principles and suggest a community effort to create a automatically as well as manually curated, generalized resource for the characterization of biological metabolites based on spectral and chromatographic data.

P077 Session 2, Thursday 4:30 - 5:30 p.m.

Plasma Eicosanoids Increase Postprandially in Normolipemic Subjects

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(1) UC Davis

Postprandial lipemia, the prolonged circulation of lipid rich lipoproteins after a meal, is considered deleterious in part because of transient endothelial dysfunction and inflammation. Plasma levels of cytokines and adhesion molecules have been shown to associate with postprandial lipemia, by as yet unknown mechanisms. Although postprandial lipemia has been shown to be modified by both long-term dietary fat composition and by acute effects of varying the lipid composition of a test meal, it is not known if factors that alter postprandial lipemia within an individual translate into differences in inflammation. Studies were conducted to measure the eicosanoids produced at different time points after consuming a 24% fat (57% omega-3 fatty acids, 17% omega-6 fatty acids, 7% saturated fat, 19% monounsaturated fat), 25% protein, 51% carbohydrate meal. Plasma was collected from three volunteers consuming the liquid meal immediately before, as well as 3 and 8 hours after the meal. Plasma concentrations of 33 eicosanoids were measured by LC/MS/MS, and ANOVA was used to determine statistically significant differences between the 0-, 3-, and 8-h timepoints. PGE2, PGD2, TXB2, EKODE, 9-oxo-ODE, and 9(10)-EpOME were significantly increased in the postprandial timepoints compared to fasting. At least a part of the inflammation associated with postprandial lipemia is promoted by the increased synthesis of inflammatory eicosanoids.

P078 Session 3, Friday 4:00 - 5:30 p.m.

Cocoa intake influence on the profiling human urinary metabolome; An open, prospective, randomized and crossover clinical trial

Rafael Llorach (1), Mireia Urpi-Sarda (1), Olga Jáuregui (1), Rosa M. Lamuela-Raventos (1), Cristina Andres-Lacueva(1)

(1) University of Barcelona

Cocoa derived foods have been shown to be a great source of health-promoting polyphenols, mainly epicatechin and catechin monomers and their polymeric procyanidins. The health effects of these polyphenols might be explained by the presence of their metabolites formed in the tissues as well as in the colon by the microbiota. Recently, Metabolomics has been proposed as powerful tool to better characterize both the intake and the effects on the metabolism of dietary polyphenols and should contribute to better understand their effects on health. The human urine metabolome modifications were explored in a randomized, crossed, and controlled experiment. Twenty one subjects consuming a single dose of cocoa as beverage containing 40 g of cocoa powder. Urine samples were obtained before consumption and during the 0–6, 6–12 and 12–24 h periods after test meal consumption. The HPLC system was coupled to a hybrid quadrupole time of flight QSTAR Elite (Applied Biosystems/MDS Sciex). Data collected from the QTOF were processed by MarkerLynx (Waters) to obtain a table of makers where each marker is defined by its retention time and exact mass and relative abundance. Multivariate data analyses were applied to the MS data by using Simca-P+ software (version 11.5; Umetrics, Umeå, Sweden). The data sets were mean centered and Pareto scaled (each variable was weighted according to 1/ SD). An unsupervised pattern recognition technique such as principal component analysis (PCA) and a supervised pattern recognition technique such as partial least squares discriminant analysis (PLS-DA) were performed. The markers obtained after the statistical analysis were identified on the basis of their exact mass which was

compared to those registered in the Human Metabolome Database (HMDB; www.hmdb.ca) and the Kyoto encyclopaedia of gene and genome (KEGG) (http://www.genome.jp/) (ΔmDa ≤ 5 mDa) using an in-house R script for R environment (Ihaka and Gentleman 1996). In addition the MS/MS experiment and comparisons with the authentic standards (when were available) where carried out to confirm the metabolite identifications.

The multivariate data analysis (PCA and PLSDA) of urine samples acquired in both negative and positive modes reveals that the individuals were well grouped for each time period showing a kinetic pattern for the evolution of the urine composition. Among the detected markers, some are fully excreted within the first 12 hrs, mainly those corresponding with the host metabolites and others are still present in the 12-24 hr period and likely correspond to microbial metabolites formed over a longer period in the colon. Concerning to the phytochemicals metabolites (food metabolome) identification, some metabolite relates with the cocoa polyphenols have been identified. In addition some endogenous metabolites have been identified using a public database queering. Furthers studies are ongoing to confirmed the metabolite identification of both exogenous and endogenous metabolites. The results showed that the metabolomics strategy allows to define new markers of cocoa consumption (exogenous metabolites) as well as to identify endogenous markers that could reflect the metabolic modifications induced by cocoa consumption. Finally, the information about the new metabolite coming from the dietary phytochemicals will be useful to evaluate the polyphenol enriched diet intake in large epidemiological studies.

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P079 Session 1, Thursday 1:00 - 2:30 p.m.

A metabolomic approach for analysing green tea

Emma Marsden-Edwards (1), Jose Castro-Perez (1), John Shockcor (1)

(1) Waters Corporation

Metabolic profiling (metabolomics/metabonomics) has emerged as a vital new area of research. Metabolic profiles contain a vast array of endogenous low-molecular weight metabolites, the composition of which depends upon the sample type (plasma urine, bile, plant extracts, etc) and factors such as the location, species, age and even the time the sample were taken. Extracting useful information from complex data sets to enable the identification of differences between samples can be extremely challenging. The data are typically comprised of a few observations and many variables. These types of data can be mined effectively using a variety of multivariate statistically methods. An efficient and robust metabolomic work-flow is described and has been used to differentiate between several different brands of Japanese green tea from various regions.

Ultra Performance Liquid Chromatography Mass Spectrometry (UPLC/MS) was used to process the comprehensive chemical compositions of the green tea extracts. The data were analysed using a program which incorporates a peak deconvolution package and collects data into a single matrix by aligning peaks with the same exact mass/retention time along with their normalised intensities was

used to identify the monoisotopic mass of the constituent components and perform multivariate statistical analyses.

Online database searching based on exact mass and elemental composition was performed to tentatively identify metabolites of interest. Structural elucidation experiments were performed using a fragmentation interpretation tool which uses systematic bond disconnections to assign fragment ions to the precursor ion, was used to confirm the putative assignments.

P080 Session 2, Thursday 4:30 - 5:30 p.m.

An untargeted nutrikinetic investigation of polyphenol metabolism in humans

Ewoud van Velzen (1), Johan Westerhuis (2), John van Duynhoven (3), Age Smilde (2)

(1) Unilever / Universiteit van Amsterdam (2) Universiteit van Amsterdam (3) Unilever

Pharmacokinetics in nutritional studies (*nutri*kinetcs) is commonly used to investigate the absorption, distribution, metabolism and excretion (ADME) of food constituents in the human metabolome with the aim to explain (or predict) health effects. These effects are often linked with temporal or longitudinal changes in the exogenous or endogenous metabolites that are present in biofluids or tissues. Nutritional and pharmacological-based intervention studies may however require different kinetic models and concepts to investigate the ADME of the molecular targets. A major concern in nutritional studies occurs when the targeted component, or its metabolized form, is already present in the normal biofluid composition. This is different from pharma-based applications with drug substances where the target metabolite is often discernible from the basal urinary constituents. Another concern is related to the characterization of the nutritional dose. In various studies the exact composition of a natural food (ingredient) is not exactly known. Also knowledge about metabolic pathways, the possible end-metabolites, and the food matrix effect is often lacking. For that reason, prior knowledge about the target metabolite and the variations between individuals is often speculative or even absent. Instead of examining the nutrikinetics of specific molecular targets, we use the NMR and GCMS urinary profiles in a holistic, metabolomics approach to assess the important endmetabolites of the human polyphenol metabolism. This pharmacokinetic approach is substantially different from classical pharmacokinetic studies where the molecular targets are known beforehand. Since the kinetic constants can be determined for each variable in the NMR spectrum and the GCMS chromatogram, an extensive amount of variables can be included in the analysis. Hence, for examining the polyphenol metabolism just a selection of the most discriminative and informative end-metabolites is relevant. A well-adapted method to assess these specific metabolites is multilevel PLS-DA. This multilevel method is particularly valuable since it is tailored to handle crossover designed multivariate data. In the current presentation, the total urinary output over 48 hours, the rate of the urinary excretion and the delay time of the selected end-metabolites were determined after black tea and grape/wine consumption. Based on these kinetic parameters, a distinction was made between subpopulations. These subpopulations are defined as being the “High and Low producer” phenotypes, the “Fast and Slow metabolizer” phenotypes and the “Fast and Slow responder” phenotypes. Heatmaps are used to define and visualize subgroups of individuals and metabolites that exhibit similarities in nutrikinetic properties.

P081 **Session 2, Thursday 4:30 - 5:30 p.m.**

Rapid and automated identification of phenolic compounds in food using LC/ESI-qQ-TOF-MS/MS and library search

Maria Gomez-Romero (1), Birgit Schneider (2), Antonio Segura-Carretero (1), Alberto Fernández-Gutiérrez (1), Gabriela Zurek (2)

(1) University of Granada (2) Bruker Daltonik

Polyphenols have great importance in the nutritional, organoleptic and commercial properties of plant-derived food and beverages. Furthermore, consumption of phenolic compounds has been associated with positive health benefits. The health effects of polyphenols depend on the amount consumed and their bioavailability. Detailed knowledge of the phenolic composition of food and beverages will contribute to a better understanding of their influence on biological properties. A method for the automated and robust identification of phenolic compounds based on accurate mass library has been developed.

The coupling of liquid chromatography and mass spectrometry (LC/MS) is an approved tool in natural products characterization. Reversed-phase chromatography and a 5min acetonitrile-water gradient were applied for the analysis of commercial standards (phenolic acids, flavonoids and some glycosides). MS measurements were performed using an ESI-Qq-TOF mass spectrometer in both ESI positive and negative mode. Acquisition was performed in automated MS/MS mode with conditions optimized for the detection of both polyphenols and their glycosides (scan range 50-800 m/z). Mass scale calibrations were performed with sodium acetate clusters. Real samples were amenable to direct analysis requiring no treatment other than filtration and dilution (phenolic concentrates were mixed with methanol during 30min).

MS and MS/MS mass spectra of common phenolic compounds were obtained using an ESI-Qq-TOF mass spectrometer, and added to the software library after careful review. Spectra were stored with all significant properties (chemical information, structure and acquisition parameters). The created library contains 250 spectra, including precursor (measured and theoretical) and fragment spectra of positive and negative polarity. Fruit juices and concentrates obtained at low temperature from vegetables rich in phenolic compounds were analyzed using the same conditions as the reference spectra. Due to the automated MS to MS/MS switching during acquisition with the user specified criteria, data of both precursor and product ions are obtained from a single run. At the end of the acquisition, the automated library search, which comprises recalibration, peak detection and settings for the library search itself, is performed. Filter criteria in the library search are e.g. the identification with MS and MS/MS spectrum, retention time, instrument settings or purity score. A report with the identified compounds is automatically generated. The quality of the identification is given by purity, fit and reverse-fit scores. The identification of the compounds can be confirmed using their mass accuracy and isotopic pattern information for formula generation.
The proposed LC/MS method is fast, sensitive, enabling the characterization of even low-abundance phenolic compounds, and provides mass accuracy and true isotopic pattern for both precursor and product ions. Furthermore, the method combines the search of retention time, MS and MS/MS spectra, which improves the identification of compounds considerably, reducing ambiguities and false positive hits.

P082 Session 1, Thursday 1:00 - 2:30 p.m.

Classification and prediction of antioxidative activities of Citrus grandis fruit extracts using 1H NMR, PCA and PLS

SOMI K. CHO (1), SEUNG-OK YANG (2), SO-HYUN KIM (2), HANA KIM (1), HYUNG-KYOON CHOI (2)

(1) Cheju National University (2) Chung-Ang University

Different parts of dangyuja (Citrus grandis Osbeck) fruits at different maturation stages were classified using a 1H-NMR-based metabolomic technique. Principal components analysis allowed the clear separation of fractions extracted with 50% methanol of different parts of dangyuja fruits at different maturation stages by combining principal components PC1 and PC2, which together accounted for 75.9% of the variance. Projections to latent structures using a partial least squares (PLS) model was used to predict the free-radical scavenging activities (FRSA) of four extracts of dangyuja fruit extracts based on their 1H-NMR spectra. The present study suggests the usefulness of combining 1H-NMR spectroscopy with multivariate statistical analysis for discriminating dangyuja fruit samples, and predicting the FRSA of different parts of dangyuja fruit samples at different stages of maturation.

P083 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolic Analysis of meju during fermentation

Hyun-Jin Kim (1), Hee Joo Kang, Min Jung Kim, Hye-Jeong Yang, Mi Jeong Sung, Jin Tak Hwang, Haeng Jeon Hur, Suk Hoo Yoon, Dae Young Kwon

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With increasing physiological and clinical knowledge of functional properties of soybean, the interest and the consumption of soy products such as tofu, soymilk, soybean paste, soy sauce, and natto are growing. Among them, soybean paste and soy sauce are traditionally made of *meju*, which is a fermented block of crushed cooked soybeans, in Korea, and their nutritional and functional properties are generally originated during the fermentation of *meju* by two primary microorganisms, *Bacillus subtilis* and *Aspergillus oryzae*. Therefore, investigation of the change of metabolic profiles of *meju* during the fermentation period is important to understand nutritional change of soy products. With enhancing the activities of enzymes including proteases and lipases originated from microorganisms, large molecules in soybean were degraded into small ones during fermentation. In particular, the contents of small peptides and free fatty acids newly produced were significantly altered with increasing fermentation period, and the profiles were analyzed by an UPLC- Q-ToF and GC/MS, respectively. Also, to compare the changes of the peptides and lipids profiles according to the fermentation, principal component analysis (PCA) was used in terms of component scores and loadings. More than five small peptides and five fatty acids quantitatively altered were found in *meju* during the fermentation, and especially, the quantity of oleic acid and linoleic acid having various functional properties increased five and ten times, respectively, by the fermentation. Also, based on PCA results about the production of these peptides and fatty acids, *meju* can be characterized depending on the fermentation period and significantly grouped by 10 or 20-day-fermentation.

P084 Session 3, Friday 4:00 - 5:30 p.m.

NMR & MS Discrimination of Wine Type and Metabolite Identification

Joshua Hicks (1), Ravikanth Veluri (1), William Brideau (1), Jonathan Wilson (2), Ali Kettani (2)

(1) Bruker-BioSpin (2) Daltonics

Wine discrimination based on metabolite profiles was carried out by Nuclear Magnetic Resonance (NMR) and Mass Specrometry (MS) to build classification models on wine types. In addition, compounds that lead to many of the variances between wines were identified. The sensitivity and robustness of NMR and MS techniques for monitoring variance in constituent compounds among different wine classes can be used for quality control, labeling requirements, fermentation monitoring, and possibly regional confirmation. Multivariate statistics were used to discriminate wine types and chemical signatures that are indicative of wine varieties. The unsupervised statistical method Principal Component Analysis (PCA) is a statistical tool used to analyze such complex datasets and reveals the distribution and characteristics of the compounds detected. NMR and MS were both able to discriminate between wine types and analysis of the variants was able to identify several compounds cross confirmed by the two methods. Several of these metabolites, such as polyphenolic polymers known as tannins, have known anti-cancer, anti-aging and antioxidant medical properties. Other, more common compounds such as glycerol, fructose, and malic acid were easily identified. Here the combined results of the two methods are presented illustrating identification and confirmation of several compounds of interest via NMR and MS techniques.

P085 Session 1, Thursday 1:00 - 2:30 p.m.

Comparison of GC-MS and NMR metabolite identification in white wines: insights into the chemical basis for wine body

Kirsten Skogerson (1), Ron Runnebaum (1), Oliver Fiehn (1)

(1) University of California, Davis

Gas chromatography-coupled mass spectrometry (GC-MS) and nuclear magnetic resonance spectroscopy (NMR) are the two most frequently used tools in metabolomic studies; both generate high-density, diverse chemical data sets, each with specific advantages and drawbacks. Few studies in the literature report independently-curated metabolite lists and resulting experimental conclusions for the same sample set with the purpose of a direct comparison of the two technologies. This study sought to compare MS- and NMR-based metabolite profiles in the characterization of white wines including varieties Chardonnay, Viognier, Pinot gris, Riesling and Sauvignon blanc.

Additionally, this work sought to identify compositional differences in these wines which correlate to the wine sensory property known as 'body'. One key attribute of any wine is its body, or viscous mouthfeel properties; despite the importance of body on the style and quality of wine, its precise origins remain unclear. Using a chemometric approach, metabolite profiles in conjunction with sensory assessments allow for the identification of individual chemical compounds correlated with sensory characteristics of a wine. Ultimately once compounds are identified, viticultural and enological practices that influence their concentrations can be elucidated and subsequently implemented to target the desired sensory characteristics.

The metabolite profiles of seventeen white wines were determined independently using GC-TOF-MS and ^{1}H-NMR. Over 300 metabolites are reliably detected in all wine samples by GC-TOF mass

spectrometry as verified by the Fiehn lab BinBase database. Over 100 of these are uniquely identified by retention index-based mass spectral libraries. One dimensional NMR data yields far fewer metabolites (~50) as identified by the commercially available software Chenomx Suite. Additionally, sensory data obtained using descriptive analysis techniques were correlated to the chemical data. Partial least squares (PLS) multivariate models were used to explain the mouthfeel viscosity rating of the seventeen white wines.

The independently calculated MS and NMR models present new insights into the chemical basis for wine mouthfeel properties. GC-MS and NMR both identified proline as highly correlated to wine body. GC-MS pointed to a potential negative correlation between fatty acids and wine body; a negative correlation between tartrate and wine body was identified by NMR and supported by GC-MS. Both technologies show potential for developing predictive models. Such models could replace sensory panels, which are expensive and time-consuming.

P086 Session 2, Thursday 4:30 - 5:30 p.m.

A search for metabolite biomarkers of potential health effects in chicken induced by organic products

Leon Coulier (1), Machteld Huber (2), Dre Nierop (3)

(1) TNO Quality of Life (2) Louis Bolk Institute (3) Muvara Statistics

In this study an attempt was made to search biomarkers to study the health effects of different feeding regimes in healthy subjects using a chicken model. The study was performed by a Dutch consortium of institutes, Louis Bolk Institute, TNO Quality of Life, RIKILT Institute of Food Safety and Wageningen University-Department of Animals Sciences, and several other institutes in the Netherlands and abroad. The study comprised a two generation, blinded animal feeding experiment with identically composed feeds from either organic or conventional products. In the second generation, metabolomics was set forth as an additional (secondary) study parameter next to a number of clinical and immunological measurements taken before and after a immunological challenge (KLH injection). The scope of the metabolomics analysis was to investigate biomarkers for organic and conventional food and to investigate whether the consumption of this food by test animals (chicken) would lead to measurable physiological differences. Metabolomics analysis was carried out on chicken plasma before and after the KLH challenge and on chicken liver using different MS-based analytical platforms. A clear distinction between the treatments could be achieved in plasma and liver. Furthermore several biologically relevant metabolites were found to react differently in the two treatments. This paper will present some of the highlights in more detail.

P087 Session 3, Friday 4:00 - 5:30 p.m.

Metabolite profiling of wine samples with LC-ESI(QTOF)MS and identifying compounds using Isotopic pattern algorithms

Ravikanth Veluri (1), Catherine Stacey (1)

(1) Bruker Daltonics

Metabolite profiling of secondary metabolites from natural sources has increased consideration due to its application in the chemotaxonomy, biomarker identification and finger printing. Polyphenolic compounds in wine are major contributors to health benefits which include reduction of risk of

cardiovascular disease, stroke and cancer. Separation of metabolites was carried out using reverse phase chromatography of the replicate runs of wine samples colleted from different regions. Liquid chromatography further coupled to Time of Flight Mass Spectrometry was proved to be nice tool for identifying Biomarkers of wine as well as comparing the distribution of the polyphenolics from different wines. Principle component analysis (PCA), a statistical tool is used to identify the compounds that are distributed differently from the complex LCMS data sets of wine samples. Identification of compounds is done with the SmartFormula3D algorithm which takes the accurate mass, true isotopic pattern and MSMS data into consideration.

LC/MS experiments were performed using a micrOTOF-Q (ESI-TOF) mass spectrometer (Bruker Daltonics, USA) coupled with an Acquity UPLC system (Waters, USA). Separations were carried out using water-acetonitrile gradients with 0.1% formic acid as modifier on a reversed-phase column (WatersC18, 1.0x50 mm, 1.7µm) at a flow rate of 0.25ml/min. Samples were diluted in 0.1% formic acid and measured in positive mode over a scan range of m/z100-1600. Each separation was calibrated using sodium formate clusters as an external calibrant.

A study of red wine extracts from different regions was carried out to understand the distribution of the polyphenolic secondary metabolites such as flavonoids, tannins, and anthocyanins. Utilization of UPLC enabled 8 minute separations significantly improving sample throughput without compromising the separation efficiency as compared to classical HPLC. Unsupervised PCA analysis of replicate analyses of different wines showed a significant clustering of the sample sets into regional groups. From the results there was a clear pattern of certain flavonoid molecules such as malvidin glucoside, proanthocyanidin B and quercetin that had a major contribution to the regional variations. These compounds were identified from their elemental composition obtained with SmartFormula which uses the SigmaFit algorithm producing a combined value of both accurate mass and isotopic pattern matching.

P088 Session 1, Thursday 1:00 - 2:30 p.m.

Multivariate analysis of beer

Sally Webb (1), Masahiro Maeda (2); Hideaki Uchida (2); Shigeki Araki (3); Toshiyuki Oshima (3); Youichi Tsuchiya (3); Katsuaki Maeda (3); Junji Watari (3); Steve Fischer (1)

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Beer is a complex beverage that has been around since antiquity. There are many different methods of production and these can affect both taste and texture. In addition, time and the environment after production affect the beer's properties. Which in turn are related its chemical composition. The analytical challenge is determine which compounds in the complex mixture are important to the beer's taste and texture.

Determining the differences between complex samples is a difficult task. Many, but not all, of the chemicals present are known, however the sheer number of compounds present make it impractical to analyze the samples by targeted MS or MS/MS alone. A fast and practical solution to this challenge is to use an untargeted analysis approach with multivariate data analysis to find these differences. The development of accurate-mass capable LC-TOF instruments greatly aids this approach by allowing simultaneous detection of all compounds with high mass accuracy.

Our study here demonstrates the utility of accurate-mass LC/MS combined with multivariate correlative analysis software to rapidly find compounds of interest in a differential analysis study.

P089 Session 2, Thursday 4:30 - 5:30 p.m.

Opening a biochemical window into nutrition with NMR-based metabolic profiling

Serge Rezzi (1), François-Pierre Martin (1), Sebastiano Collino (1), Emma Peré-Trepat (1), Ivan Montoliu Roura (1)

(1) Nestlé Research Center

The development of postgenomic technologies, i.e. transcriptomics, proteomics and metabonomics, has revolutionized our vision of nutrition. Metabonomics provides an original approach to assess physiological regulatory processes of a living organism at the system level via the mathematical mining of metabolic profiles. Based on the holistic profiling of multiple metabolic entities in biological matrices, the metabolic profiles encapsulate the biochemistry of the complex molecular interactions of host with its microbial partners and environmental factors. Therefore, it is a suitable approach to understand the complex relationship with foods and nutrition.

Metabonomic analysis can be conducted with different analytical techniques. Proton Nuclear Magnetic Resonance (1H-NMR) spectroscopy is a cornerstone of metabonomic technology that provides a fast, reproducible, and non-destructive snapshot of multiple metabolites in biological matrices without or minimum sample preparations. The high dimensionality of metabolic profiles is explored with multivariate analysis techniques to recover key metabolic variations indicative of specific physiological processes including pre-pathological states, nutritional interventions, and trans-genomic cross talk between host and microbial partners. In this presentation, NMR-based metabolic profiling techniques of biofluids and tissues, including lipidomic analysis, will be reviewed in the context of nutrition research. A modelling of biochemical events in aging mice with a multicompartment NMR profiling of biofluids and tissue extracts will be presented. Furthermore, a particular emphasis on the modulation of gut microbiome–host metabolic interactions with probiotic (Lactobacillus paracasei or L. rhamnosus) and prebiotic (galactosyloligosaccharides) supplementation will be made. Additionally, the metabonomic approach will be finally discussed for personalized nutritional management.

P090 Session 3, Friday 4:00 - 5:30 p.m.

Lipidomics in studies of life style associated diseases Part 2: biological interpretation

Suzan Wopereis (1), J. Bouwman (1)

(1) TNO Quality of Life, Zeist

Life style associated diseases like obesity, diabetes and cardiovascular disease are rapidly becoming the worldwide number 1 health problem. In developing strategies for preventing or treating life style associated pathologies, it is critical to identify the key metabolic changes involved in the development of these diseases. These metabolic changes should be responsive to nutritional and pharmaceutical intervention to allow their evaluation. Lipids play an important role in metabolic stress, inflammation and oxidation - processes that often form the basis of these life style associated diseases. TNO developed several lipidomics platforms that can be applied in nutritional and pharmacological studies (presented in Part 1 of this poster, see I. Bobeldijk et al.)

These different lipidomics platforms were successfully applied to livers from a mouse study involving 3 different diet interventions: a control diet, a high fat diet of plant origin and a high fat diet of

animal origin. Both high fat diets were found to cause metabolic syndrome with insulin resistance. Interestingly, the high fat diet of plant origin resulted in a significant higher degree of whole body insulin resistance than the high fat diet of animal origin, which was also reflected in the hepatic lipidome of these animals.

This poster (Part 2) will provide an overview of the most striking differences found in the hepatic lipidome of mice on the two different high fat diets. We show that livers of mice on plant based high fat diet contained higher contents of pro-inflammatory eicosanoid precursors and of total diacylglycerol. Furthermore, livers of mice on animal based high fat diet contained lower contents of ceramide species. We will identify feasible mechanisms that might be the key in the development of a different insulin resistance phenotype and what in the diet might have caused this difference.

P091 Session 1, Thursday 1:00 - 2:30 p.m.

Metabonomics and Its Potential Application for Canine and Feline Nutrition

Ziad Ramadan

Apologies for the missing abstract, file was corrupted in the database

P092 Session 2, Thursday 4:30 - 5:30 p.m.

A lipidomics approach to investigate lipid metabolism in diabetic dbdb mice

Claire Boursier-Neyret (1), Thierry Umbdenstock (1), Vincent Croixmarie (1), Gilles Simonin (1), Nathalie Landerouin (1)

(1) TECHNOLOGIE SERVIER

Among the different antidiabetic drugs developed for the treatment of Type II Diabetes, the PPARg agonists have been widely studied. However, the secondary effects observed with this class of agonists, such as oedema, weight gain, or more severe effects such as hepatotoxicity or nephrotoxicity, are important to assess before clinical development. The availability of a predictive animal model of Type II Diabetes but also of secondary effects of PPARg agonists is therefore crucial for studying the development of this class of compounds.

In the current study, a diabetic dbdb mouse model was used, with the PPARg agonist Rosiglitazone as antidiabetic treatment. The potential secondary effects linked to lipid changes were assessed by the determination of different classes of Lipids in tissues, namely Liver, White Adipose Tissue and Plasma. The Lipid changes were monitored by either specific quantification of Free fatty Acids (FFA) and Triglycerides (TG) in the corresponding tissues or a more global approach (Metabonomics approach) focusing on entire families of FFA and Triglycerides, i.e. Lipidomics analysis of FFA and TG in the same tissues by UPLC-TOF or Trap-MS and/or NMR. For LC-TOF-MS Triglycerides determination, data interpretation based on their individual profile, namely the number of Carbon atoms and number of unsaturations for the different Triglycerides has been developed, allowing to differentiate not only the quantitative changes in TG but also the qualitative differences in TG profiles between db+, dbdb control and dbdb treated mice.

The major differences were observed in the liver for both TG and FFA, with a drastic increase of Lipids in the Liver after Rosiglitazone treatment, and to a lesser extent in WAT. A decrease of Lipids was concomitantly observed in plasma, in accordance with the mechanism of action of PPARg

agonists, namely increase of lipids in liver, storage in adipose tissues and decrease in the circulation. A dose effect of Rosiglitazone was also evidenced, mainly in the Liver. A parallel transcriptomics analysis of genes involved in the lipid metabolism has been performed. The major changes were related to the synthesis and transport of FFA and TG, in relation with the lipidomics data observed. This study confirmed the interest of combining new and targeted Omics tools for characterising pharmacological models, and are seen as a prerequisite before screening any novel PPARg agonists and their potential effects on Lipid metabolism.

P093 Session 3, Friday 4:00 - 5:30 p.m.

Fluxomics: application of stable isotopes to study the effect of body fat distribution on the physiological response to a dietary fat intervention.

Elwin Verheij (1), Kitty Verhoeckx (1), Wouter Vaes (1), Albert de Graaf (1), Andreas Freidig (1)

(1) TNO Quality of Life

The analysis of metabolite concentrations and their changes upon treatment(s) is a powerful approach in many settings. Unfortunately metabolomics is not a 'one size fits all' tool. Investigating the dynamics of physiological responses to treatments is aided by the measurement of metabolite fluxes. This is even more evident in nutrition trials if treatment and the metabolome overlap. The intelligent use of stable isotopes is an elegant method for ADME and flux analysis.

This paper describes the application of LC-MS and GC-IRMS to investigate the physiological response in light obese male volunteers (BMI ~ 29) to a dietary fat intervention as a function of body fat distribution (upper body obese – UBO and lower body obese -LBO). Carbon-13 labeled medium chain fatty acids and long chain fatty acids were used to investigate the metabolic fate of MCFA and LCFA as function of diet adjustments and UBO/LBO. Plasma and adipose tissue lipids were analyzed with GC-IRMS to determine the enrichment of fatty acids. The results provided interesting information on changes in fatty acid metabolism as function of diet adjustments and obesity type.

In the same experiment d3-leucine was administered (i.v.) to measure the de novo production of ApoB100 and Adiponectin. Two different methods were applied. ApoB100 was isolated by lipoprotein fractionation followed by hydrolysis and LC-HRMS analysis of leucine and d3-leucine (LTQ Orbitrap). Adiponectin was isolated from plasma using antibodies, and subsequently digested with trypsin. Deuterium enrichment of the resulting leucine containing tryptic peptides was measured by LC-MS analysis of the adiponectin peptide map. This paper describes the methodology applied and some first results will be presented.

P094 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolic Profiling of human serum in diabetic nephropathy using ^{1}H NMR and LC-MS/MS

Eun Young Kim (1), Ji-Young Yang (1), Mi-Ryung Kim (2), Chan-Wha Kim (2), Geum-Sook Hwang (1)

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Diabetic nephropathy is a renal disease which arises as the consequence of diabetes. DN is also one of the most significant long-term complications in terms of morbidity and mortality for individual diabetes patients. Clinical management and therapeutic intervention from the earlier stage of

diabetic nephropathy (DN) is of major importance to prevent the progression of DN to end stage renal disease.

In this study, we investigated the serum metabolic changes in normal human and diabetic nephropathy patient in order to study metabolic profiling and to discover the potential biomarkers. First, we investigated the serum metabolite profiles using ^{1}NMR and multivariate data analysis (MVDA) and identified the several different metabolites in diabetic nephropathy compared with normal control, including 2-hydroxybutyrate, 2-hydroxyisovalerate, creatinine, leucine, dimethylamine, methylamine, myo-inositol, glucose, and pyruvate. Then, the potential biomarkers of diabetic nephropathy, which were observed by ^{1}H NMR, were validated using liquid chromatography-mass spectrometry. Targeted metabolite analysis by LC-MS was carried out with a quadrupole mass spectrometer equipped with an electrospray (ESI) source as LC-MS/MS interface. The results of this study indicate NMR-based global metabolic analysis can yield a broad array of biomarkers for effects of diabetic nephropathy and target metabolite analysis by LC-MS provides the quantitative conformation of biomarker candidates.

P095 Session 2, Thursday 4:30 - 5:30 p.m.

NMR metabonomics to investigate obesity and growth disorders – from the lab into the clinic

Horst Joachim Schirra (1), Shaffinaz Abd Rahman (1), Tony Hyunh (2), University of Queensland (1), Gary Leong (1)

(1) University of Queensland (2) Mater Children's Hospital

A major hindrance in metabolomics research is the presence of a large fraction of unknown metabolites detected in complex biological samples when purified chemical standards are unavailable and metabolite databases remain incomplete. Herein, we demonstrate an integrative strategy for de novo identification and quantification of metabolites using capillary electrophoresis-electrospray ionization-mass spectrometry based on fundamental electrokinetic, thermodynamic and molecular properties of an ion. Unambiguous identification of metabolites was realized in this work based on accurate prediction of relative migration times of putative metabolite candidates using computer electrophoretic simulations. A model set of cationic metabolites was also used to determine the relative ionization response of various amino acids, amines and peptides, including structural isomers. Predicted calibration curves were generated for reliable metabolite quantification without commercial standards as demonstrated by metabolite recovery experiments in red blood cell lysates. Virtual metabolomics offers a promising way to identify and quantify newly discovered biomarkers and drug metabolites while minimizing time-consuming chemical synthesis, purification and univariate calibration protocols that are required for rapidly expanding metabolomic initiatives.

P096 Session 3, Friday 4:00 - 5:30 p.m.

Fatty acid ethanolamines, glycines and monoacyl-glycerol profiling in the plasma, muscle and adipose of the Zucker rat

John Newman (1), Anthony Thomas (2), Sean Adams (1), Katrin Georgi (2)

(1) USDA Western Human Nutrition Research Center (2) University of California Davis

Background: Endocannabinoid production in peripheral tissues is responsive to leptindependent signaling and constitutes a primary regulator of tissue growth, cell differentiation, and whole-body energy balance. Recent investigation of these lipophilic mediators has made it apparent, however, that the unsaturated mono-acyl glycerols, fatty acid ethanolamines and related lipid amides, represent an array of potent endogenous ligands of multiple membrane bound receptors. These include the cannabinoid receptors (pain and satiety perception, pro-hypotensive), the vanilloid receptors (pain perception, anxiety), and the peroxisome proliferator activated receptors (adipogenesis, fatty acid metabolism, inflammation). Moreover, these receptors each show unique specificity to structurally similar members of these chemical classes. As such, profiles of fatty amides and acyl glycerols provide information-dense outputs relevant to energy balance and whole animal physiology.

Methods: A sensitive positive mode electrospray ionization HPLC/MS/MS method has been developed to quantify 32 fatty amides and glyceryl esters from biological samples. Briefly, rat tissues (~100mg) are spiked with deuterated surrogates, homogenized in cold ethanol, diluted with water, and isolated with solid-phase extraction (SPE) on an HLB stationary phase (Waters). Analytes are separated by reverse phase (100 x 2.1 mm, 1.8 µm, Zorbax Eclipse C18, Agilent) with a flow rate of 400µL/min and a gradient elution with a mobile phase of acetonitrile, formic acid and ammonium acetate.

Results: Limits of detection ranged from 20 fmol on column for amides to 200 fmol on column for the glyceryl esters. This method was able to routinely detect oleoyl and arachidonoyl glycine, 1- and 2- glyceryl-esters of oleate, linoleates, and arachidonate, and a host of saturated, monounsaturated and polyunsaturated fatty acid ethanolamines in the plasma, muscle, and adipose tissues of lean and obese Zucker rats. Oxygenated arachidonoyl ethanolamines (e.g. 15-HETE-ethanolamine) were not detected in peripheral tissues at these concentrations. Changes in plasma and muscle fatty ethanolamine and mono-acyl glycerols were positively correlated, but were distinct from adipose tissue changes. While adipose tissue declines in multiple very long chain fatty acid ethanolamines were detected in obesity, the dihomo-gamma-linolenic acid ethanolamine increased in all tissues measured.

Conclusion: Using a novel targeted metabolic profiling method, we have confirmed that a defect in central leptin sensing has unique impacts on the peripheral fatty ethanolamine and monoacyl acyl-glycerol levels in the plasma, muscle, and adipose tissue of the rat. As such, the described fatty amide and acyl-glycerol assay provides a tool for the investigation of cell growth and differentiation, systemic energy balance, inflammation and pain regulation in cells, whole animals, and human subjects. Moreover, the physical chemical behavior of these compounds, and the ultimate method allowing their quantification, suggests that this metabolic profile can be determined in extracts produced for the quantitative analysis of oxylipids.

P097 Session 1, Thursday 1:00 - 2:30 p.m.

Adipose PPARg Activation Ameliorates Insulin Resistance through Anti-inflammatory Functions

Shigeki Sugii (1), Peter Olson (2), Annette Atkins (3), Steven Watkins (4), Ronald Evans (1)

(1) Howard Hughes Medical Institute and The Salk Institute for Biological Studies (2) University of California, San Francisco (3) The Salk Institute for Biological Studies (4) Lipomics Technologies, Inc.

Activation of peroxisome proliferator-activated receptor gamma (PPARγ) through its high affinity ligands, Thiazolidinediones (TZDs), is a widely used strategy to treat type 2 diabetes. It remains largely undefined, however, how receptor activation in individual tissues contributes to TZD's insulin-sensitizing action. Here we demonstrate that in the setting of diet-induced obesity, mice expressing a constitutively active PPARγ selectively in mature adipocytes exhibit enhanced insulin sensitivity and glucose tolerance to a similar degree as TZD-treated wild-type mice. Adipose PPARγ activation leads to improvement of insulin resistance in liver and muscle. In addition to modulation of genes in lipid and carbohydrate metabolism, significant changes were found in immune system and inflammatory processes in both TZD-treated and transgenic tissues. The transgenic mice exhibit reduced macrophage infiltration and increased insulin signaling in adipose tissue, but not enhanced adipogenesis, suggesting that the insulin-sensitizing effects of TZDs are uncoupled from the receptor's adipogenic functions. In addition, metabolomics analyses revealed specific metabolite changes that may mediate the downstream anti-inflammatory effects. Collectively, these data demonstrate that adipocyte-specific PPARγ activation is sufficient to improve systemic insulin sensitivity through anti-inflammatory properties and recapitulates TZD action in the diabetic state.

P098 Session 2, Thursday 4:30 - 5:30 p.m.

Activity of hepatic stearoyl-coa desaturase 1: key to difference in whole body insulin resistance?

Suzan Wopereis (1), J. Bouwman (1)

(1) TNO, Quality of Life

The development of diet-induced obesity with insulin resistance was investigated in the APOE3Leiden mouse model. The mice were divided in three experimental groups (control diet, standard chow; high-fat diet Lard (HFL), 45 energy % lard; and high fat diet Palm oil (HFP), 45 energy % palm oil). The apoE3Leiden mice on both high fat diets developed obesity with insulin resistance (confirmed with CLAMP analysis at week 16). Interestingly, the mice on the HFP diet developed a significant higher degree of insulin resistance than the mice on the HFL diet.

Liver lipidomics was performed on day 1 and 3 and weeks 1, 2, 4, 8, 12 and 16 (n=5 per time point / group). Multivariate and univariate statistics showed that several significant changes could be determined in fatty acid and lipid metabolism. Three active phases of hepatic lipidome response could be determined in the mice on both high fat diets. Furthermore, multivariate statistics revealed that more similarities than differences existed between the hepatic lipidomes of mice on the two high fat diets. However, a few lipids showed some specific changes in one of the two high fat diets. These differences suggested a more pronounced inflammatory state in livers from mice on the HFP diet. The most obvious difference, however, was the linear increase of the ratio between monounsaturated and saturated palmitoyl and stearoyl lysophosphatidylcholines - suggesting increased activity of hepatic stearoyl coA desaturase 1 (SCD-1)- in mice on HFL, whereas normal in mice on HFP. From literature it is clear that SCD-1 activity is connected to the development of insulin resistance, although the exact mechanism is still unclear. This poster will provide possible

mechanisms why increased hepatic activity of SCD-1 is the key to milder whole body insulin resistance in mice on high fat diets.

P098-2 Session 3, Friday 4:00 - 5:30 p.m.

Metabolomics Platform: A Useful Tool for the Study of Diabetes and Metabolic Disorders

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The Metabolomics Platform is a joint research facility created by URV (Universitat Rovira i Virgili, Tarragona, Spain) and CIBERDEM (Spanish Biomedical Research Network in Diabetes and Associated Metabolic Disorders). The main goal of the Metabolomics Platform is to offer metabolomic services to the biomedical and clinical research groups from CIBERDEM and URV.
The main vision of the Metabolomics Platform is not only act as a measurement facility but to offer metabolic consulting, trying to get fully involved in the metabolic-related experiments proposed by the groups. This means that the collaboration should start from the very beginning of the study (definition of goals, dimension and characteristics of the sample set, metabolic experimental design) until the very end (data processing and interpretation), helping to obtain sound, significant and useful clinical results for the different research groups willing to use the facility services.

The equipment available in high field NMR allows high throughput analytical measurements of body fluids i.e., serum, urine as well as tissue or biopsies analysis of humans and/or animal models (i.e., rats, mice, etc.). Starting in 2009, the platform will be able to offer Chromatographic and Mass Spectrometry services as complementary tools for metabolomic studies. Data treatment will be carried out mainly with the AMIX® software package. It comes with the NMRMetaPro® database, which allows the identification of those metabolites included and characterized in such a complete database (with around 500 substances). The Metabolomics Platform will also uses Matlab ®, a very flexible mathematical environment that allows to apply many statistical and pattern recognition algorithms that are starting to be used in metabolomics and/or other -omic sciences.

The use of advanced statistical, chemometric, multivariate and artificial intelligence algorithms will allow turning large measurement datasets into useful clinical information. The diagnosis of metabolic related illnesses and risk evaluation on large population studies, are the major concerns of the platform together with the identification of metabolic pathways or involved biomarkers in metabolic related diseases.
Although specially addressed to the needs of CIBERDEM and URV groups, the Metabolomics Platform welcomes any biomedical research group to contact its members for scientific collaboration proposals or/and metabolomic services.

1 http://www.metabolomicsplatform.com/index.html

P098-3 Session 2, Thursday 4:30 - 5:30 p.m.

The NMR metabonomic approach to explore the metabolic phenotype in genetically modified mice

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A preliminary metabonomic study using 1H NMR spectroscopy has been applied to explore the role of monocyte chemoattractant protein-1 (MCP-1) in the liver aqueous extract obtained from LDLr deficient mice. Previous results described an important role of MCP-1 in metabolism in response to both hyperlipidemia and diet, suggesting a dual role, metabolic and immunological, for this chemokine. Male LDLr-deficient mice (LDLr-/-) and MCP-1LDLr-deficient mice (MCP1-/-LDLr-/-), with the same genetic background (C57BL/6J), were used for this study. Mice were randomly assigned to two dietary groups; one was fed with regular chow and the other with chow supplemented with palm oil (20% fat w/w) and 0.25 % (w/w) cholesterol.
In the current study, high resolution 1H NMR spectroscopy -based metabonomic approach has been applied to understand the metabolite consequences of MCP-1 expression in the liver tissue metabolism in response to both hyperlipidemia and high-fat and cholesterol diet.
The loading plot of PCA model showed the NMR chemical shifts that were responsible for separation of the data along factor 2 attributed to MCP 1 deficiency. Thus, in the liver aqueous extract, MCP1-/-LDLr-/- mice showed higher concentrations of taurine and lower concentrations of lactate and glucose than their counterpart LDLr-/- mice on both diets but the effect on hepatic taurine concentration was less evident in animals fed the high-fat, high-cholesterol diet.
Our data not only indicates that MCP-1 expression is especially important in the lipid and glucose liver metabolism but also, this analysis leads us to establish a link between MCP-1 expression and taurine concentration. These results are in accordance with recent findings indicating that the dietary ingestion of taurine may have antioxidant activity reducing the production of inflammatory mediators such as MCP-1.

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P099 Session 3, Friday 4:00 - 5:30 p.m.

Metabolic profiling of drug responses in acute myeloid leukaemia cell lines

Stefano Tiziani (1), Alessia Lodi (1), Farhat Khanim (1), Mark Viant (1), Christopher Bunce (1)

(1) University of Birmingham

The acute myeloid leukaemias (AMLs) are a genetically heterogeneous group of cancers characterized by the abnormal maturation, survival and proliferation of myeloid cells in bone

marrow. Despite improved chemotherapy (approximately 50 to 75% of adults achieve complete remission) only about 25% of patients remain disease-free for a prolonged time. A large majority of patients succumb due to persistent or relapsed AML. A panel of methods has been used to assess the effects of new drugs which should be antileukaemic without being myeloablative or systemically toxic in cell based models. The current study builds on preceding work showing the antileukaemic actions of several steroidal and non-steroidal drugs against AML cells. We have employed 1H NMR metabolomics to identify phenotypical differences between three AML cell lines (K562, KG1a, and HL-60) and to distinguish subtle changes in metabolite concentrations induced by acute exposures to the lipid lowering drug Bezafibrate (BEZ) and the contraceptive steroid medroxyprogesterone acetate (MPA). MPA inhibits AKR1C3, a protein which has been linked to PPARγ and NF-κB by regulating 15dΔ12,14-PGJ2 availability. Bezafibrate has been shown to have a pronounced effect on the production of reactive oxygen species (ROS) through multiple pathways including mitochondrial damage and transcriptional changes. The increase of oxidative stress triggered by steroidal and non-steroidal drugs plays a key role on mitochondrial dysfunction in cancer cells. In this study we have observed a sustained oxidative stress in all the three cell lines, leading to an accumulation of succinate and acetate. In addition to these common trends, in HL-60, oxaloacetate decarboxylation seems to promote the accumulation of malonate which, in turn, can cause the lowered fumarate level by inhibition of the succinate dehydrogenase activity. We also show a pronounced synergistic effect of the combined administration of drugs which target independent mechanisms with a joint functional end point. These effects were distinctly different for cell lines which differentiate upon treatment vs others which apoptose. This study shows the potential of 1H NMR based metabolomics as a tool to profile drug responses in cancer cell lines. The observed effects represent mechanistically relevant changes induced by treatment of AML cell lines with BEZ and MPA, indicative of a strong link between ROS and Krebs cycle control.

P100 Session 1, Thursday 1:00 - 2:30 p.m.

Discrimination and prediction of Korean vs. Chinese herbal medicines by OPLS-DA based on 1H-NMR metabolomics.

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(1) Inha University, (2) Seoul National University

Correct indication and discrimination of origins of herbal medical products are becoming increasingly important with recent interests in alternative medicine. To develop an efficient tool for discrimination and prediction of Korean vs. Chinese herbal medicines, we employed NMR-based metabolomics approach combined with OPLS-DA multivariate analysis. Using *Scutellariae bicalensis* as an initial model system, we performed NMR spectral acquisition and analysis, identifying a number of metabolites. Holistic statistical data analysis with OPLS-DA approach gave cleaner separation between Korean and Chinese than more conventional PCA-based approach. Analysis of the STOCSY spectrum revealed citric acid and arginine as key metabolites underlying the difference between the Korean and Chinese samples. As a stringent validation of the statistical model, we also performed blind prediction tests of the sample origins using external test set. Our model correctly predicted the origins of all 11 test data set, demonstrating its robustness. We tested the wider applicability of the developed method for the prediction of origins for three other herbal medicine from Korea and China and obtained near-perfect accuracy in predicting the unknown samples. The solid statistical validity and prediction results shown here for four herbal medicine suggest that our NMR and OPLS-DA based metabolomics approach could also be applied to developing tools for origin discrimination for other plant materials.

P101 Session 2, Thursday 4:30 - 5:30 p.m.

Principal Component Analysis of Isotopomer Data Reveals Low Flux Correlation from Plasma [U-13C6]-D-Glucose to Cholesterol in Valproate Induced Liver Toxicity in Rats

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(1) United States Food and Drug Administration (2) SiDMAP, LLC

The mammalian liver is central to glucose disposal into circulating plasma triglycerides and cholesterol, therefore decreased glucose dependent cholesterol synthesis may constitute a simple marker to assess liver function and drug-induced liver toxicity. Principal Component Analysis (PCA) was applied to precursor  product 13C isotope enrichment data in this study to correlate glucose derived acetate's contribution to plasma cholesterol after a single toxic valproate dose in rats. Female CD-1 mice were injected subcutaneously with saline (n=8) or 600 mg/kg valproic acid (VPA) (n=8); 12 hours later, they were injected with an intraperitoneal (I.P.) dose of 0.5 g/kg [U-13C6]-D-glucose. Cholesterol 13C labeling and acetyl-CoA enrichment from plasma glucose in the regression equation was modeled as corresponding constants' random variables representing unexplained variation in the dependent variable as the null hypothesis. Regression statistics, analysis of variance (ANOVA) and linear regression were generated using the best fit model to assess the decrease in least squares as the primary criteria for VPA toxicity. Time dependent plasma [U-13C6]-D-glucose fractions were 50.85%, 24.89% and 13.24% at 60, 120 and 180 minutes, respectively, after saline, or 51.89%, 26.66% and 15.34% after VPA treatment. Glucose isotopomers produced via glucose dependent carboxylation, TCA cycle anaplerosis, triose and hexose cycling were unaffected in the VPA treated group compared to saline. On the other hand, while 13C labeled fractions of plasma cholesterol were 2.6%, 2.0% and 1.6% at 60, 120 and 180 minutes after saline treatment with a Multiple R of 0.9972, R Square of 0.9944 and Adjusted R Square of 0.9889 [Standard Error 0.0528], VPA induced a less glucose responsive 2.2%, 2.2% and 1.8% cholesterol labeling curve with a Multiple R of 0.6951 R Square of 0.4831 and Adjusted R Square of -0.0337 [Standard Error 0.2069]. The 13C acetyl-CoA enrichment saturation curve from glucose to plasma cholesterol was also significantly disrupted by VPA treatment with a Multiple R of 0.5547 (Control 0.9994), R Square of 0.3076 (Control 0.9989) and Adjusted R Square of -0.3847 (Control 0.9977) [Standard Error 22.0186]. In summary, lower order principal components of mass isotopomer data derived from a 13C labeled substrate, namely glucose as the precursor in this study, towards single or multiple products, such as cholesterol synthesis and transport from the liver, contain probably the most important and sensitive aspects of metabolomics data with regard of the liver's response to toxic drugs. Evenly maintained tracer precursor uptake and synthesis of certain groups of metabolites via hepatic production (i.e.: glucose and lactate) with an altered kinetics in isotope labeling in key metabolites of the fatty acid and cholesterol transport chain serve as early, sensitive and robust markers of single dose drug toxicity using Principal Component Analysis.

P102 Session 3, Friday 4:00 - 5:30 p.m.

Using a non-targeted metabolomics workflow and statistical analysis of LC/MS/MS data to obtain a metabolite profile of Nefazadone

Jeffrey Miller, Johnie Brown, James Ferguson

(1) Applied Biosystems

Recent reporting has indicated that incorporating either a non-targeted approach followed by powerful data filtering, or by taking advantage of quadrupole mass filters to increase sensitivity (in a targeted approach) are both very useful workflows for investigators to find and characterize possible metabolites of drug candidates[1,2].

Here we employ a new strategy using the unique scanning functions and power of two mass spectrometers; a hybrid quadrupole linear ion trap (4000 QTRAP system) and a quadrupole-time-of-flight (QSTAR Elite) to screen, detect and characterize *in vitro* metabolites of nefazodone as a study model. Nefazodone (incubated with human liver microsomes), and a control were injected several times to obtain statistical data sets for mining. The data sets were processed with MarkerView™ 1.2 software to obtain a list of candidates based upon changes in the two sample sets by T-test and principal component analysis (PCA). The software was used to filter out the significant possible metabolites and confirm their relevance by accurate mass or structural similarities using the MS/MS fragmentation.

Results show that both mass spectrometer platforms yielded very similar lists of possible metabolites, approx. 35-40, ranging from major metabolites down to very low-level (~0.1%) species. The MS/MS fragmentation data obtained by the experimental design, allows the researcher to further delve into the data and apply structural tools to confirm compound identity by library matching with standard databases.

We believe this methodology will be important in the lead optimization of drug candidates in the drug discovery process, as well as useful in the quality assurance programs characterization of impurity profiling and degradation analysis of compounds for in several industries.

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P103 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolomics analysis of urine and kidney tissue in rats treated with Valproate

Moti Rosenstock (1), Kurt Boudonck (2)

(1) Teva Pharmaceutical Industries Ltd (2) Metabolon Inc

Valproate (VPA) is a drug widely used to treat epilepsy, but it has serious adverse effects including hepatotoxicity, teratogenicity and antifolate activity. In order to better understand its mechanism of action and toxicity profile, a globally unbiased metabolomics analysis was performed. Sprague-

Dawley Crl:CD(SD) rats were treated with VPA (500 mg/kg) or saline (vehicle) once daily for 1, 5 or 28 days. Urine and kidney tissue samples were collected from groups of 6 rats each (12 rats for vehicle group) at days 1, 5 and 28. Full data curation of kidney tissue samples yielded 547 metabolites, and urine yielded 657 metabolites, consisting of amino acid metabolites, peptides, carbohydrates, lipids, energy metabolites, nucleotides, cofactors, vitamins and xenobiotics. Over 130 metabolites were found to be significantly different in urine between vehicle and VPA-treated groups at each time point. In kidney tissue, over 50 metabolites were significantly different at each time point. Treatment with VPA induced increased excretion of carboxylic acids such as beta-hydroxyisovalerate, 3-methylcrotonylglycine and 2-methylbutyrylglycine, indicative of an inhibition of acyl-CoA dehydrogenases or acyl-CoA carboxylases by VPA. VPA also caused increased excretion of acetylated amino acids, consistent with an effect of the drug on deacetylases or acetyltransferases. Furthermore, several metabolites from the omega-oxidation pathway were increased as well in urine from VPA-treated animals, which is likely a compensation mechanims for decreased beta-oxidation. Finally, several bile acids were increased in the kidney tissue of the VPA group, indicating that VPA may have induced some liver toxicity. In summary, we have demonstrated that a global unbiased metabolomics analysis can provide information on the mechanism of action of a drug, as well as on toxicity issues, in one and the same analysis.

P104 Session 2, Thursday 4:30 - 5:30 p.m.

Drug Metabolism in 3-D tissue engineered liver microreactor

Yu Zeng (1), Joseph Moritz (2), Ju Liu (3), Walker Inman (1), Steven Tannenbaum (1)

(1) Massachusetts Institute of Technology (2) BASF Corp (3) BG Medicine

Perfused microvasculature has been established in our 3D hepatocyte culture microreactor system, the LiverChip, to develop a reliable model to conduct assays for toxicology and metabolism for human disease. This multi-well plate bioreactor system fosters 3D organization of liver cells and provides local microscale perfusion, creating a high throughput cell culture system that allows a higher level of physiological mimics of human and animal tissues. Therefore, this feature maintains more microevironmental *in vivo* aspects than other 2D or 3D culture models.

To evaluate the liver function of this system, fundamental studies have been conducted to determine whether physiological ratios of enzymes (CYP) exist in culture isolated rat primary hepatocytes in the microreactor. Two probe substrates for CYP 3A1/3A2 (midazolam - MID) and CYP 1A1 (Ethoxyresorufin-O-deethylation - EROD) were used in this study. A rapid quantification LC/MS assay using single quadrupole mass spectrometer combined with UPLC chromatography was developed to analyze the clearance of the probe substrates. A model of flow and reaction in the system was used to extend the kinetic parameters from 3D cultures. Using this method, the intrinsic clearance was estimated for the probe substrates in the isolated hepatocytes, 2D Matrigel™ overlay and 3D microperfused culture. The data indicate that the average enzyme ratio is near that of freshly isolated hepatocytes. Thus, this model may be useful in the evaluation of liver toxicity and drug development.

P105 Session 3, Friday 4:00 - 5:30 p.m.

Predictive metabolomics of human CSF

Anna Wuolikainen (1), Peter Andersen (1), Thomas Moritz (2), Stefan Marklund (1), Henrik Antti (1)

(1) Umeå University (2) Swedish University of Agricultural Science

A strategy for reliable detection of metabolic markers termed *predictive metabolomics* has been applied and evaluated for human cerebrospinal fluid (CSF). We have investigated non-disease related factors suspected to induce changes in metabolite concentration and metabolite composition of CSF samples. CSF was collected *via* lumbar puncture in a case-control study based on 13 male patients with different neurological conditions. The CSF handling and storage was carried out based on a statistical experimental design scheme with the factors and factor settings being: storage temperature (-80/-20°C), type of collection tube (polypropylene/polystyrene), time before freezing (0, 10, 30, 90, 150 min) and diagnosis. Gas chromatography-time of flight mass spectrometry (GC/TOFMS) was used to characterize the CSF samples from 12 of the 13 patients, while samples from one patient were characterized using nuclear magnetic resonance (1HNMR). Chemometric methods based on the OPLS methodology were used to evaluate the metabolic variation in the data. The results provide clear evidence that alterations in sample handling procedures significantly affect the metabolic composition of human CSF samples, where storage temperature turned out to be the factor influencing the metabolic concentrations the most. The presented results will hopefully contribute to a better understanding of the CSF metabolome; present praxis for high quality CSF sampling and sample handling for metabolomics.

P106 Session 1, Thursday 1:00 - 2:30 p.m.

In vivo HR-MAS of Zebrafish Embryos for NMR-based Metabonomics

Axel Meissner (1), Shuning He (2), Eberhard Humpfer (3), Sibel Goraler (1), Herman P. Spaink (2)

(1) Leiden University Medical Center (2) Leiden University (3) Bruker BioSpin GmbH

Zebrafish are well suited for studies in genetics, embryology, development, and cell biology since they exhibit unique characteristics, including ease of maintenance and drug administration, short reproductive cycle, and transparency of the embryos that permits visual assessment of developing cells and organs. In view of the fact that the toxic response in Zebrafish is comparable to results in mice, Zebrafish embryos have gained importance as vertebrate model for assessing drug effects and toxicity. Generally Zebrafish bioassays are cheaper and faster than mouse assays, and therefore suitable for high throughput screening.

Effects of many xenobiotics have been studied by endogenous enzyme assays or visual examinations of the organ morphology; in addition MRI approaches have been used on Zebrafish embryos as well as adult Zebrafish. However, no high resolution nuclear magnetic resonance in-vivo data on Zebrafish has been reported so far. In this paper we present the application of *in-vivo* HR-MAS NMR spectroscopy on Zebrafish embryos. The feasibility of this method for high throughput (toxicity) screening using NMR-based Metabonomics methodology is discussed and an example for evaluation of a human disease model is given.

P107 Session 2, Thursday 4:30 - 5:30 p.m.

Identification of perturbations in human metabolism due to propionic acidaemia, using metabolomics approach

Carools Reinecke (1), Japie Mienie (1), Elardus Erasmus (1), Gerhard Koekemoer (1)

(1) North-West University

Propionic acidaemia (PA) is a life-threatening inborn error of metabolism with autosomal recessive inheritance, caused by deficiency of propionyl CoA carboxylase (PCC, EC 6.4.1.3). One biochemical feature of PCC deficiency is the formation of an extensive array of secondary metabolites from branched chain amino acids, an affected Krebs cycle metabolism, and clinical manifestations, like ketoacidosis, and hyperammonemia and aberrations in other minor metabolic pathways. A classic question in PA is whether a consistent metabolite profile with a high diagnostic value exists in this disorder[1]. Contemporary hyphenated chromatographic methods, like GC-MS, followed by second-order bioinformatics data analysis now provide powerful tools for profiling of such inherited biochemical disorders. In this report we present results on such an analysis, using urine samples as analytical material, obtained from a group of patients diagnosed with propionic acidaemia (18 cases) and a comparative group of controls (19 cases).

Organic acids from urine samples were extracted and analysed with GC-MS methods according to our standardised protocol[2]. Analysis of all peaks in the GC chromatogram was done through the Agilent system, and MS identification of each component was done on a dedicated database for detection of metabolites and biomarkers of inherited disorders. All components were subsequently expressed as mg component per mg creatinine (Cr) to compensate for differences in concentration between various urine samples. These figures formed the variants for the statistical analysis, for which we construct a matrix for bioinformatics analysis from the 37 cases and a variable space with 464 dimensions. The bioinformatics analysis and transformation of this data set is presented in an accompanying presentation[3].

PCA of the transformed data set illustrated differences in the metabolite profiles, where each of the two groups occupied discrete areas on the scores map of the first two principal components. Using a partial least squares-discriminant analysis (PLS-DA), an improved grouping of controls and patients could be shown in a score plot of the metabolites as independent variables, and the first 20 most important variables (minor metabolites) for this grouping could thus be identified. Finally a hierarchical cluster analysis was performed using Ward's method with a squared Euclidian distance measure. The variables used to cluster items were the first 20 important biomarkers, and the result of Ward's method can be displayed as a dendogram. From this analysis it became clear that the additional biomarkers identified can be used to classify the cases either as controls or patients. Moreover, two sub-categories of patients could be identified through this approach.

Conclusions:

1. This proof-of-concept study demonstrates that metabolomics can expand the range of organic acids used for diagnosis of PA. These results are complementary to a recent LC-TOF analysis of the hydrophilic metabolites and carnitine conjugates associated with PA
2. It is also shown that metabolomics could distinguish between the phenotype (metabolite profiles) of patients suffering from PA, which may be useful for patient clinical evaluation, and which might hold promise for treatment through therapeutic interventions.

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P108 Session 3, Friday 4:00 - 5:30 p.m.

Application of NMR-Based Metabolomics to Male Reproduction Using Rhesus Macaques

Ching Yu Lin (1), Pei-Hsuan Hung(2), Lutz Froenicke (2), Leslie Lyons (2), Marion Miller (2)

(1) National Taiwan University (2) University of California, Davis

Sperm metabolome was used to study male reproduction in a non-human primate model, rhesus macaque (*Macaca mulatta*). We used ^{1}H nuclear magnetic resonance (NMR)-based metabolomics to characterize sperm energy supply and metabolic responses to environmental pollutants in rhesus monkey. The intension of the sperm energy supply study was to examine whether the ATP source of rhesus sperm was primarily from glycolysis or mitochondrial oxidative phosphorylation. Rhesus sperm was treated with alpha-Chlorohydrin (ACH, 3-chloro-1,2-propanediol), which is a known inhibitor of sperm glycolysis, or pentachlorophenol (PCP), which is an uncoupler of oxidative phosphorylation, or a control media *in vitro*. ^{1}H-NMR spectroscopy following principal component analysis was used to characterize the metabolite profiles of sperm. We have found that at the dose level used, no observable metabolic difference between control and PCP treated samples. However, metabolic effects of ACH were consistent with inhibition of glycolysis with decreased lactate and ATP. The study shows that either the primate sperm is insensitive to uncoupling agents of oxidative phosphorylation or the glycolysis plays a more important role in maintaining sperm ATP levels. We have also discovered that metabolic variances due to seasonal differences. A higher level of formate was seen in the fall and higher levels of carnitine and acetylcarnitine were observed in the spring as well as possible differences in lipoprotein content.

The second study examined the metabolic effects of Environmental Tobacco Smoke (ETS) *in vivo* on rhesus sperm. We also compared the metabolic impacts with semen quality and sperm function. Adult rhesus macaques were exposed to ETS *in vivo* for six months. Semen samples were collected before, during, and after the exposure for evaluation. ^{1}H NMR following principal component analysis was applied to examine the metabolic effects of ETS on sperm. While ETS exposure did not affect semen quality and sperm function, there were metabolic turbulences in sperm collected during and after the ETS exposure. ATP, formate, acetylcarnitine, and citrate were decreased once monkeys exposed to ETS. When ETS exposure stops, there is a shifting between carnitine and acetylcarnitine level.

In both studies, we have demonstrated the metabolomic application to male reproduction with a potential to solve the problem of male infertility. Our study also illustrated that metabolomic approach is more sensitive and able to better understand the underlying mechanisms of action of toxic compounds in the environment.

P109 Session 1, Thursday 1:00 - 2:30 p.m.

^{1}H NMR metabolomics study of age profiling in children

Haiwei Gu (1), Zhengzheng Pan (1), Narasimhamurthy Shanaiah (1), Nagana Gowda (1), Daniel Raftery (1)

(1) Purdue University

Metabolic profiling of urine provides a fingerprint of personalized endogenous metabolite markers that correlate to a number of factors such as gender, disease, diet, toxicity, medication, and age. In order to unravel the contributions of each factor, it is important to study these factors individually if possible. In this study, age-related metabolic changes in children of age 12 years and below were analyzed by ^{1}H NMR spectroscopy of urine. The effect of age on the urinary metabolite profile was observed as a distinct age-dependent clustering even from the unsupervised principle component analysis. Further analysis using partial least squares with orthogonal signal correction regression with respect to age, resulted in the identification of an age related metabolic profile. Metabolites that correlated with the age included creatinine, creatine, glycine, betaine, TMAO, citrate, succinate, and acetone. These results may be potentially useful in assessing the biological age (as opposed to chronological) of young humans as well as in providing a deeper understanding of the confounding factors in metabolomics applications.

P110 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolomics as a potential tool for tuberculosis diagnosis

Du Toit Loots (1), Gerhard Koekemoer (1), Carools Reineck (1)

(1) North-West University

The dual epidemics of HIV infection and multidrug-resistant (MDR) tuberculosis (TB) currently threaten global TB control by either preventing or prolonging the already time consuming diagnostic tests used for TB diagnostics. Current TB diagnostics rest on the use of sputum smears and culturing for identifying active disease. These methods, although widely used, lack sensitivity and specificity, and in the case of culturing, take 6-8 weeks to obtain a diagnostic result. MDR-TB is an additional threat to successful TB control, as its diagnosis is currently based on culturing, in addition to drug susceptibility testing, with results taking as long as 2 or more months to obtain. The above mentioned diagnostic tests are further compromised by co-infection with HIV. Considering this, faster and more sensitive diagnostic tools are essential for identifying active TB and MDR-TB, especially in the light of the growing HIV/AIDS epidemic (Getahun et al. 2007).

Using Metabolomics as a tool for diagnosis is novel in the sense that it could potentially identify all species specific and non-specific metabolites and considers the ratios of these to one another (hence all end products of gene expression) in an untargeted manner. These differences can be analyzed using hyphenated mass spectrometry (MS) apparatus (e.g. GC/MS) in a matter of hours, and can hence be used to accurately identify and differentiate various organisms with speed, precision and sensitivity. In order to analyze the vast amounts of species specific data generated by such an analysis, statistical data manipulation is applied though a process defined as Bioinformatics. Consequently, it is not unrealistic to assume that such an approach can be used for disease diagnosis, disease characterization and specific disease biomarker identification. This relatively new approach, using hyphenated MS equipment, Metabolomics and Bioinformatics, has recently been used by us at the Centre of Metabolomics at the North-West University (NWU), for the rapid,

specific, sensitive disease diagnosis and disease bio-marker identification of various inborn metabolic diseases in humans. Using the same diagnostic approach, it is realistic to assume, based on the nature of the chemical compounds which the infectious bacteria and infected host produce in the lungs, that this method may also be used for TB and MDR-TB diagnostics using patient collected sputum samples. Preliminary results from our collaborative group in The Netherlands have confirmed this using headspace analysis of sputum samples on an electronic nose. What makes this approach unique, is that it has the potential to differentiate active TB, MDR-TB and other infectious organisms or combinations of these, in a single analytical run taking no longer than 2 hours. In this poster we will present an outline for the development of a more effective detection procedure of TB, based on both previously completed experimental research and theoretical information on TB. This will be presented in a manner to stimulate discussion on experimental design involving the use of Metabolomics for diagnostic applications.

P111 Session 3, Friday 4:00 - 5:30 p.m.

Flexibility in energy metabolism supports hypoxia tolerance in Drosophila flight muscle: metabolomic and computational systems analysis.

Laurence Coquin (1), Jacob Feala (2), Andrew McCulloch (2), Giovanni Paternostro (1)

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The fruit fly Drosophila melanogaster offers promise as a genetically tractable model for studying adaptation to hypoxia at the cellular level, but the metabolic basis for extreme hypoxia tolerance in flies is not well known. Using 1H NMR spectroscopy, metabolomic profiles were collected under hypoxia. Accumulation of lactate, alanine, and acetate suggested that these are the major end products of anaerobic metabolism in the fly.A constraint-based model of ATP-producing pathways was built using the annotated genome, existing models, and the literature. Simulations supported the hypothesis that the ability to flexibly convert pyruvate to these three byproducts might convey hypoxia tolerance by improving the ATP/H+ ratio and the efficiency of, glucose utilization. Traditional enzymatic assay are used to determine the pool of glycogen, trehalose and ATP, in order to refine the model. Metabolomic profile of selected mutants have also been collected, to determine the pathways used to produce alanine and acetate as well as to better understand the biological effects of these metabolites.

P112 Session 1, Thursday 1:00 - 2:30 p.m.

Etiological diagnosis of pneumonia through a urinary metabolic profile

Hao Fu (1), Kathryn Rankin (1), Andrew Cheypesh (1), David Chang (2), Paige Lacy (1)

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Pneumonia is a term used to describe an acute or chronic infection of the lower respiratory tract caused by any of a number of pathogens including bacteria, viruses, fungi and parasites. Diagnosis of pneumonia is complicated by the fact that non-infectious diseases such as congestive heart failure, pulmonary infarction, vasculitis or drug reactions, can mimic the disease. Although significant advances in medicine have been made, pneumonia remains the leading cause of death by infection in developed countries mainly due to the difficulty in promptly identifying the etiologic agent. In practice, the pathogen responsible for pneumonia can only be determined in 15 – 20% cases. The

major causative pathogen of pneumonia is Streptococcus pneumoniae, which is present transiently in the throat and upper respiratory tract of approximately 40% of the population. Diagnosis of pneumococcal pneumonia is typically made by positive blood or sputum culture in the presence of a compatible clinical picture. However, only 6-10% of blood cultures and 11% of sputum cultures yield S. pneumoniae, and results are rarely available within 36 hours.

In this study, we use nuclear magnetic resonance spectroscopy to quantitatively measure the profile of metabolites excreted in the urine of patients with pneumonia caused by S. pneumoniae and other microbes in a multicenter trial. We found the urinary metabolomic profile for pneumococcal pneumonia was significantly different from those without pneumonia as well as those with viral and other bacterial forms of pneumonia. Furthermore, we found that we could elicit a similar metabolic response in mice upon intratracheal infection with S. pneumoniae. These results demonstrate that urinary metabolomics may be useful for the effective diagnosis of CAP, and may pave the way for improved and robust diagnostic tests for CAP.

P113 Session 2, Thursday 4:30 - 5:30 p.m.

Multidimensional analytical strategies for *schistosoma mansoni* infection in the mouse model

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Schistosomiasis is a widespread parasitic disease and a severe debilitating illness. It is estimated that around 200 million people are infected and early diagnosis and treatment is crucial to avoid chronic granuloma infection.

A metabolomics platform comprising NMR, UPLC-MS and CE together with multivariate data analysis was applied to find biomarkers of infection. Analyses were performed on urine samples collected from 10 mice infected with 80 cercariae over a period of 53 days and the equivalent number of control animals during the same time span.NMR fingerprints for the infected animals showed an increase in several metabolites with the highest discriminant metabolites being phenylacetylglycine and *p*-cresol glucuronide. UPLC-MS provided a urine signature where infected animals predominantly showed an increase in phenylacetyl glycine and other gut microflora metabolites such as *p*-cresol glucuronide and sulphate. Multivariate analysis of CE data provided a similar result classifying five main markers including phenylacetyl glycine for infected animals.

The three techniques employed have shown phenylacetyl glycine as the most discriminant metabolite after 48 days of the infection. When correlation techniques were applied to phenylacetyl glycine, we found that this was positively correlated to three metabolites in its same pathway, phenylalanine, hydroxyphenylacetic acid and phenylacetic acid. With all three analytical techniques and increase in differentiation between control and infected was observed with time corresponding to increasing severity of infection.

The results of the present study have highlighted dominant biomarkers, this validates their potential as early diagnostic targets in urine for the treatment of this parasitic disease.

P114 Session 3, Friday 4:00 - 5:30 p.m.

Metabolomic and proteomic profiling of drug exposed human HaCaT cells to provide an insight into psoriasis pathogenesis.

Katherine A Hollywood (1) , Catherine L Winder (1) , Warwick B Dunn (1) , David Broadhurst (10 , Susan Slack (1)

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Psoriasis is a common dermatological disorder characterised by areas of dry, thickened and reddened skin. There is no cure for psoriasis although a number of treatments are currently available to ease symptoms exhibited by the sufferer; however, side effects and low impact rates are present. Three well established anti-psoriatic drugs were chosen to be investigated within this work; Dithranol, Methotrexate and Cyclosporin A.

In this study we have used a spontaneously transformed human keratinocyte cell line, HaCaT, as a model system to study psoriasis. HaCaT cells were cultured in Dulbecco's modification of Eagle's media (DMEM) containing 10% fetal calf serum, L-glutamine and penicillin/streptomycin. The cells were maintained at 37°C with 5% CO2 and grown to approximately 85-90% confluence. A range of drug concentrations corresponding to that of the therapeutic dose were applied for 24h and the subsequent effect analysed.

Metabolomic analysis of the cell's footprint (exometabolome) and fingerprint (internal metabolome) was conducted via GC-MS. Used growth media was aspirated; syringe filtered and snap frozen for metabolite footprint analysis. Cells were washed with ice cold PBS. Ice cold MeOH (100%) was applied to quench metabolism prior to harvesting through scraping. Extraction of metabolites from within the HaCaT cells was conducted through three freeze-thaw cycles. The MeOH sample was freeze dried with an internal standard, derivatised and GC-MS analysis performed. Data were analysed by multivariate techniques.

Proteomic analysis incorporated two analysis techniques. One approach utilised the robustness of HaCaT cells, growing them directly onto steel MALDI target plates. Similarly to above cells where grown in tissue culture dishes until approximately 85-90% confluence followed by drug treatment for 24h. Sample preparation for MALDI-ToF-MS analysis was minimised to drying the sample for 10min at 50°C followed by pipette spotting of matrix. The MALDI matrix employed for analysis was Sinipinic acid. MALDI profiles where collected and data analysed by multivariate techniques. The second approach was proteomic analysis through 2D gel electrophoresis followed by MALDI-ToF-MS analysis of differential spots (that is to say, spots that were significantly different between the drug treatment and control). Identified protein masses where searched against the Mascot protein databases.

P115 Session 1, Thursday 1:00 - 2:30 p.m.

Fourier Transform infrared micro-spectroscopy in the study of Keloid disease

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(1) University of Manchester

Keloid scars are believed to result from abnormal wound healing and are characterized by persistent growth of wound tissue after re-epithelialisation and extension of scar tissue beyond the original

borders of the wound. At present there is limited understanding regarding the exact pathogenesis of the disease and this could be aided by improved knowledge of the chemical composition of the scar tissue.

A total of six histologically proven keloid scars were excised from four individual patients and FT-IR data collected from 10µm sections using single point collection and area mapping (64 x 64 pixels = 267µm x 267µm). All data presented within this study were collected using a Bruker Hyperion 3000 (Bruker Optics) IR microscope coupled to a Bruker Equinox 55 FT-IR spectrometer (Bruker Optics). The IR microscope was equipped with a mercury cadmium telluride (MCT) and a focal plane array (FPA) detector and connected to a manually controlled x-y stage. Data acquisition was conducted using Bruker OPUS software and analysis performed within MATLAB 7.1.

22 line mapping experiments produced 154 spectra from the analysis of the six individual keloid slices. Line mapping was conducted in both vertical and horizontal directions. Vertical line maps represented a transition from epidermis, through normal dermis region (termed Grenz zone), into the diseased keloid part (superficial edge; keloid centre; deep edge) and returning to normal dermis region again. Similarly, line mapping in a horizontal direction transfers from a normal dermis region, onwards into the diseased keloid part (peripheral edge; keloid centre; peripheral edge) and finally returning into normal dermis. In this way it was possible to categorise our measurements in the following categories, epidermis (e), normal (n), keloid (k) or uncertain (u). Uncertain regions were regions that could not be assigned without an element of doubt from interpretation of corresponding H&E staining or light image from the microscope.

FT-IR image analysis was conducted on the section that best represented a keloidal specimen. A vertical line image was collected by collating 14 individual FPA images (each FPA = 64x64 IR spectra). The areas were selected using the light images provided by the microscope and aligned by manual co-ordination of the x-y stage. The line map incorporates a transition from normal epidermis to dermal keloidal areas and returning to normal skin morphology.

A combination of multivariate analysis and partial least square regression were applied to the collected data. Through principal components analysis it has been shown that diseased and normal tissue can be clearly distinguished. Additionally the production of collated line images can visually present the key variations in the chemical composition of keloidal versus normal skin morphology. This technique can be considered a successful method for the classification of diseased and normal tissue in a keloid patient and shows promise as a transferable technique for the detection of other skin diseases.

P116 Session 2, Thursday 4:30 - 5:30 p.m.

Global Survey of Hepatic Disease-associated Metabolic Phenotype in Human Serum

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Every cell in the body leaves a record of its physiological state in the products it sheds to the blood, either as waste or as signals to neighboring cells. We previously described use of capillary electrophoresis with electrospray ionization time-of-flight mass spectrometry (CE-TOFMS) for metabolic biomarker discovery in mice serum (*1*). Mass spectrometry instruments with increased detection sensitivity, together with "first draft" of human metabolome (*2, 3*) and data analysis tools (*4*) have facilitated large-scale screening of metabolic phenotypes. In the present study, we used our screening platform to interrogate human serum. We collected and tested serum samples of healthy

control and patients with hepatic cirrhosis and hepatic cancer. We took advantage of the durability of the CE system and optimized fragmentor, skimmer, and Oct RFV voltages in order to extend *m/z* range for more comprehensive screening. Good reproducibility, linearity, and sensitivity were obtained, indicating that the normalized peak-area/internal-standard ratio was highly reproducible and reflects the change in relative abundance of particular metabolites throughout the study. We globally detected >1,450 peaks in serum and highlighted multiple changes in metabolite levels including not only known markers (tyrosine, branched chain amino acids and bile acid) but alsonew biomarker candidates. We also built a tree-based serotype classifier using relative metabolite abundance data of the samples. Transparent classifier based on multiple metabolites constructed from the dataset showed very high accuracy in an independent blind test-set. Our CE-TOFMS-based platform can thus provide insights into the disease-associated metabolic phenotype on a large scale and serve as a powerful new tool for discovering low molecular weight biomarkers.

1. T. Soga *et al.*, *J Biol Chem* 281, 16768 (2006).
2. D. S. Wishart *et al.*, *Nucleic Acids Res* 35, D521 (2007).
3. E. Marshall, *Science* 315, 583 (2007).
4. R. Baran, M. Robert, M. Suematsu, T. Soga, M. Tomita, *BMC Bioinformatics* 8, 72 (2007).

P117 Session 3, Friday 4:00 - 5:30 p.m.

Quantitative metabolic signatures of steroids to investigate biological changes in prostate diseases

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Abnormalities in steroid hormones synthesized from cholesterol in the adrenal cortex, ovaries, and testes are responsible for development and prevention of many diseases including cancer. Due to their biochemical roles in endocrine system, the quantitative evaluation of steroid hormones is needed to elucidate altered expression of steroids. A quantitative analysis by gas chromatography-mass spectrometry (GC-MS) has been achieved for comprehensive profiling assay of 70 endogenous urinary steroids including 22 androgens, 18 estrogens, 15 corticoids, 13 progestines, and 2 sterols. The GC-MS based urinary steroid assay has been validated and applied to both benign prostatic hyperplasia (BPH) and prostate cancer (PC) to evaluate altered metabolic patterns compared to healthy subjects. This method showed good separation and quantitative results for all steroids monitored within a 50-min analytical running in the selected ion-monitoring mode. The quantitative results obtained from both 19 patients with PC and 59 patients with BPH were compared with 41 age and sex-matched healthy subjects. Urinary ratio of 5□-androstane-3□,17□-diol to androstenedione (or testosterone) was significantly higher in patients with prostate cancer than in both normal controls and BPH patients, while 5□-androstan-3□, 17□-diol to dihydrotestosterone ($P < 0.001$) was present in much lower levels than both cases. Multi-targeted profiling analysis of steroid hormones generates quantitative results to explain activities of enzymes correlated with steroidogenesis and their metabolic signatures manipulated by multivariate data analysis may be a useful tool for clinical diagnosis as well as mining biomarker in hormone-dependent diseases.

P118 Session 1, Thursday 1:00 - 2:30 p.m.

Deciphering the metabolome of the versatile pathogen *Staphylococcus aureus*

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(1) University of Greifswald

Staphylococcus aureus, a human pathogen bacterium that resist in communities on body surfaces, present on one third of the world population, cause infections even in healthy individuals by invading tissues and fighting against the host immune system. This bacterium has evolved resistance to many antibiotics and therefore representing a significant health care concern.

We introduce here a metabolomic platform to cover most of the entire metabolome for the *S. aureus* strain COL grown in chemical defined medium. Using complementary methods to detect the highly chemically diverse groups of metabolites, e.g. GC-MS, LC-MS, ^{1}H-NMR gives a comprehensive insight into the metabolome of *S. aureus*. The sample protocol was tested for common pitfalls in metabolomic studies, like quenching effectiveness (energy charge), leakage and recovery of metabolites.

Our results for the sample workup protocol indicating that fast filtering, quenching with liquid nitrogen in cold ethanol and subsequently cell disruption with glas beats is a reliable procedure for *S. aureus* COL metabolite extraction. We identified more than 100 metabolites with the described analytic platform in *S. aureus* COL and a huge amount of unknown compounds was extracted reproducible. To full fill data demands from systems biologists, we started with absolute metabolite quantification of main compounds in *S. aureus*. The obtained data are consistent with previously published results from proteome[1] and transcriptome[2] studies.

[1] Kohler, C., Wolff, S., Albrecht, D., Fuchs, S., Becher, D., Buttner, K., Engelmann, S. and Hecker, M., 2005. Proteome analyses of Staphylococcus aureus in growing and non-growing cells: A physiological approach. International Journal of Medical Microbiology 295, 547-565.
[2] Fuchs, S., Pane-Farre, J., Kohler, C., Hecker, M. and Engelmann, S., 2007. Anaerobic gene expression in Staphylococcus aureus. Journal of Bacteriology 189, 4275-4289.

P119 Session 2, Thursday 4:30 - 5:30 p.m.

Qualitative and quantitative limitations on positive/negative switching analysis on a UPLC-LTQ-Orbitrap

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Over the long term, the successful integration of metabolomics into laboratory, pharmaceutical, and clinical workflows would be greatly aided by increasing throughput. Modern chromatographic workflows and faster mass spectrometers have aided this effort, while simultaneously increasing precision – an ideal combination. The ability of mass spectrometers to examine both positive and negative ionization modes would seem to offer a similar ability to increase throughput at little cost. A counter concern is that such approaches might compromise performance by having fewer scans available for quantitation, by giving less useful Ms^n scans, or by using suboptimal buffers, We directly tested this using an analytical replicate run 24 times, 8 under each mode. Qualitative and quantitative data suggests that the gain of time associated with running the instrument in a switching mode is not worth the lost of precision.

P120 Session 3, Friday 4:00 - 5:30 p.m.

The Human Serum Metabolome

David Hau (1), Jun Peng (1), Jianguo Xia (1), Bijaya Gautama (1), Dan Tzur (1)

(1) University of Alberta

Continued advances in detection and separation analytical technologies have augmented the interest in comprehensive metabolic profiling of biofluids and tissues, and have highlighted the need for developing comprehensive reference resources. As part of our effort to systematically characterize the human metabolome, we currently present a global analysis of the human serum metabolome.

For this holistic analysis, high-resolution Nuclear Magnetic Resonance (NMR) spectroscopy and Gas Chromatography–Mass Spectrometry (GC–MS) with computer-aided literature mining were combined to identify and quantify the metabolites that can be commonly detected (with today's technology) in the human serum metabolome. Samples from healthy individuals and various patients were collected for the NMR analysis, whereas pooled blood serum samples were prepared for the LC–MS and GC–MS studies. Data acquisition and analysis was performed according to SOP and in-house developed protocols. Our literature survey was facilitated by several computational tools developed for the Human Metabolome Database (http://www.hmdb.ca) and the in-house text-mining tool called PolySearch was used (http://wishart.biology.ualberta.ca/polysearch/).

Our experimental results indicated that global metabolic profiling methods can routinely detect 350 different compounds in serum. With NMR spectroscopy 44 compounds were detected and quantified, whereas GC–MS methods could detect and quantify 312 compounds. Tables containing the compounds, concentrations, spectra, protocols and links to disease associations that we have found for the human serum metabolome are presented.

P121 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolomic screening of human plasma using U-HPLC coupled to a Bench-top Non-hybrid Orbitrap Mass Spectrometer with HCD fragmentation

Donna Wilson (1), Xiaofeng Guo (2), Sucharita Dutta (1), Jens Hoefkens (3), Bruce Kristal (2)

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Metabolomics is the comprehensive and quantitative analysis of wide arrays of endogenous metabolites in biological samples. These numerous analytes have very diverse chemistries and polarities occurring at different abundance levels within complex matrices. Metabolomic screening of numerous technical and biological replicates is crucial for results to be statistically and biologically meaningful. Therefore, discovery phase metabolomics relies on rapid full scan analysis to measure as many metabolites as possible. However it is impossible to detect and quantify chemical species which are not adequately mass resolved. One current screening approach utilizes ToF instruments coupled to UHPLC delivering high mass accuracy (~5ppm) at a maximum mass resolution of <15,000. The inability to detect ions with mass resolution of at least 50,000 can lead to inaccurate mass measurements caused by unresolved background matrix interferences.

In this work we highlight a full mass scan screening approach using a novel single stage orbitrap mass spectrometer coupled to UHPLC, capable of providing high mass accuracy at a range of resolutions: 10,000, 25,000, 50,000 and 100,000. Chromatography was performed using an Accela U-HPLC equipped with a 2.1 mm id Hypersil Gold C18 column packed with 1.9 um particles at a flow rate of 600 uL/min and 40°C column heating.

The analysis focused on the detection and quantification of low molecular weight components of human plasma. U-HPLC coupled with a small particle column afforded a fast analysis time while maintaining very high chromatographic resolution (peak width <3 seconds at half height). The mass accuracy data (mass difference less than 2 ppm with external mass calibration) was used to confirm elemental composition. Identification of several compounds was facilitated by using HCD fragmentation, while also enabling semi-quantitative determinations.

Comparison of the measurements at resolution of up to 50,000 clearly indicated the need for higher resolution for screening applications in complex matrices. This is because interferences may not be resolved from the analytes and in many cases the use of resolutions between 25,000 and 50,000 resulted in poor mass accuracies (> 10 ppm). In contrast, acquisition of data at 100,000 resolution yielded excellent mass accuracies (<2 ppm) for the vast majority of the measured compounds. As a consequence selectivity and also sensitivity is increased. The new single stage orbitrap mass spectrometer is able to perform this high resolution with a fast scan repetition rate making it compatible with fast chromatography. This work shows that a combination from uHPLC and high resolution mass spectrometry provides a rigorous analytical methodology for metabolomic screening and overcomes limitations of using resolution of less than 50,000.

P122 Session 2, Thursday 4:30 - 5:30 p.m.

High througput high resolution lipidomics platform

Elwin Verheij (1), Ivana Bobeldijk-Pastorova (1), Leon Coulier (1)

(1) TNO Quality of Life

The lipidome is a subset of the full metabolom. Despite being just a subset, the complexity makes it a daunting challenge for analytical chemists. It is simply impossible to address the full lipidome with a single method because of the large physical property and concentration differences of the various lipid classes. This can only be achieved by combining multiple methods, each giving only one or more pieces of the puzzle, but together the full picture is obtained.

The lipidomics platform consists of several LC-MS, LC-MS/MS (SRM), GC-MS, UPLC-MS and Direct Infusion Nanospray FTMS methods in combination with lipid extraction/fractionation approaches. Major drawbacks of multiple methods are the large sample volume requirement, low throughput and high cost, which needed to be addressed in the development process. This paper discusses the development of this mixed technology lipidomics platform, with emphasis on high throughput techniques such as UPLC and Direct Infusion Nanospray. Automated processing of direct infusion data is a critical issue which will be discussed in this paper. The performance of the platform and its modules will be illustrated by means of applications in nutrigenomics, toxicogenomics and pharmacogenomics.

P123 Session 3, Friday 4:00 - 5:30 p.m.

Comprehensive chromatographic approach to LC-MS based non-targeted urinary metabolomic profiling.

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In LC-MS based metabolomic profiling much of the emphasis is given on the complementary stationary phase selection like reversed phase and HILIC columns but using routine mobile phases. In present study, we have demonstrated the how use of different mobile phases on reversed phase C18 column can be used to maximize the coverage of urinary metabolome and also used HILIC (Hydrophilic Interaction Chromatography) column to retain polar metabolites present in urine. We have applied this methodology on urine samples from kidney transplant patient. Until now there are very few well established biomarkers to monitor kidney function or dysfunction and there is much need for more specific biomarkers. The wealth of urine metabolome likely to provide more metabolite markers for even more precise and reliable diagnosis of kidney function and transplant. Urine is in intimate contact with kidney and contains vast range of endogenous metabolites hence best suited to study kidney function. Human urine contains several classes of compounds like organic acids, amino acids, amines, purines, pyrimidines, sugar alcohols. All these compounds have diverse physical and chemical properties in solution like different pKa, pH, solubility, polarity, hydrophobicity, dissociation and ionization constant (Ka), which affects their chromatographic as well as electrospray ionization behavior immensely. Hence to cover this chemical diversity of urinary metabolome comprehensive chromatographic approach is required with not only with different stationary phases but also with different mobile phases to separate and to detect maximum number of metabolites. In several compounds ionization occurs in mobile phase itself, hence mobile phase pH, buffer and solvents, is critical for formation and detection of metabolite ions.

Method: Urine samples from ten kidney transplant patients were obtained 2 days before and after (5days, 30days) of kidney transplant. Samples were diluted in twice amount of mixture of acetonitrile for protein precipitation and extraction. Chromatographic separations were performed by reversed phase C18 column on Thermo linear ion trap LC-MS using three different mobile phases at acidic, basic and mid pH range with 35 min. long gradient. And same samples were also analyzed on UPLC-HILIC column by using Waters UPLC-MS/MS system. All four experiments were performed separately and data acquired as full scan mode in mass range of 100-1000Da in ESI positive mode. For identification of detected ions data-dependent MS/MSn scans function were performed.

All spectral raw data of above experiments was processed by using freely available software XCMS for peak detection, alignment and normalization. The PCA plots were obtained by using SIMCA-P+ software. These scores plots were used for visualization of time parametric changes in metabolite ion patterns before and after kidney transplant and corresponding loading plots were used to determine significant feature from this data.

Results: Use of different mobile phases resulted in detection of different ions and differential chromatographic patterns from urine samples of kidney transplant patients and healthy subjects. Score plots show good separation among urine samples from healthy subjects and from patients; and also among patient samples before and after kidney transplantation.

Thus, identification of significant metabolite ions from this data can give biomarkers to study and monitor kidney transplantation and this comprehensive chromatographic approach to LC-MS based urinary metabolomic profiling proves its potential application to study kidney function.

P124 Session 1, Thursday 1:00 - 2:30 p.m.

GC/APCI-TOF MS: a valuable addition to the metabolomics toolbox

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(1) LUMC Leiden (2) Bruker Daltonik

Gas chromatography-mass spectrometry (GC/MS) is one of the most important and widely used techniques in analytical chemistry. Unsurpassed separation power makes it a method of choice for analysis of any type of complex chemical mixtures. With the emerging of metabolomics concept, GC and GC/MS quickly developed to an essential tool of the new discipline. Indeed the combination of GC with MS, providing the possibility to reveal a chemical identity of studied compounds on basis of retention time and mass information, is an extremely powerful tool. However, Electron Ionisation (EI) and Chemical Ionisation (CI) conventionally used in GC-MS are relatively harsh ionisation techniques. Even though many commercial and open source databases are available, the fragmentation of the compounds is sometimes so strong that it impairs the structural significance of the parent ion. Here, we present an alternative, namely - Atmospheric Pressure Chemical Ionisation (APCI) in combination with orthogonal TOF-MS, which provides excellent mass accuracy and a wide dynamic range. The calculation of the analytical parameters (linearity, and limit of detection) and validation of the method were made with a standard mix composed by 35 compounds covering a wide range with regard to polarity and molecular weight characteristic for body fluids metabolites. Derivatization reaction was based on a two-step procedure: methoxyamination and silylation. The samples were then injected onto a HP-5-MS column (30 m, 0.25 mm ID, 0.25 mm film) and analyzed by a temperature gradient of 5ºC/min over 57 min (oven initial T= 70ºC kept over 5 min). As a next step, we applied the developed method to the analysis of human cerebrospinal fluid (CSF) samples. A complex chromatographic pattern with 300 or more peaks was obtained. Finally, we discuss possible strategies for compound identifications and the feasibility of the GC/APCI-TOF-MS for biomedical applications.

P125 Session 1, Thursday 1:00 - 2:30 p.m.

Novel API-Qq-TOF mass spectrometer with ultra-high mass resolution and mass accuracy at high scan speed

Oliver Raether (1), Melvin Park (1), Sebastian Goetz (1), Gabriela Zurek Armin Holle (1)

(1) Bruker Daltonik

The combination of MS/MS capabilities with mass resolution and mass accuracy has made API-Qq-TOF MS instruments an indispensable tool for many different applications ranging from molecular formula generation to the profiling of highly complex samples in proteomics and metabolomics. In past years, new detector and digitizer technology enabled a wide intra-spectra dynamic range of more than four orders of magnitude. Accurate mass and preservation of the isotopic pattern made confident automated formula generation in MS and MS/MS possible. However, identifying and quantifying trace compounds based on accurate mass LC/MS in complex matrices requires much higher resolution.

The goal of this work was the improvement of mass resolution without compromising other performance factors like sensitivity, dynamic range, mass accuracy or scan speed. For this purpose, the effective flight path has been extended and several ion optical elements like ion guide, focusing

optics and collision cell have been improved. The performance factors (resolution, mass accuracy, limit of quantitation, limit of detection, limit of identification, dynamic range) have been tested systematically for several applications: identification of trace compounds from synthetic chemistry, metabolite profiling studies, intact proteins work, or label-free quantitation with complete digested cell lysates as matrix.

As result of the new design we could improve mass accuracy and mass resolution in MS and MS/MS by factors. In fact, mass accuracy and limit of identification of compounds in trace amounts in complex matrices could be improved dramatically, because of the much higher resolution compared to currently available API-Qq-TOF mass spectrometers. We were able to quantify and identify much more regulated peptides and proteins in proteomics applications. Also the limit of detection and quantitation as well as the effective dynamic range for quantitation has been improved substantially. For metabolite profiling studies this could be also shown for very narrow chromatographic peaks, achievable with an ultra high pressure HPLC system because of the possible high acquisition speed of 20 spectra per second.

P126 Session 1, Thursday 1:00 - 2:30 p.m.

Hybrid LC/MS/NMR as a unique tool for analysis of highly complex samples and integrated chemical identification

Gabriela Zurek (1), Aiko Barsch (1), Carsten Baessmann (1), Hartmut Schaefer (2), Manfred Spraul (2)

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One of the most challenging tasks in analytical chemistry today is the characterization of complex biological mixtures, i.e. for biomarker discovery, or the discrimination of disease states, origin etc. LC/MS is an established tool for high throughput analysis with good sensitivity at reasonable costs. NMR is the most prominent analytical technique for structure elucidation and in screening for e.g., metabolomics or routine applications like food and beverages analysis. The combination of LC/MS and Flow-NMR, however, can effectively yield qualitative and quantitative information by applying statistical tools. Data will be shown such as newborn metabolomics and the geographical discrimination of beverages by (bio)marker detection.

The identification of new chemical entities (NCE) is a routine task in most analytical labs, yet it may still require a high effort in data collection and in particular interpretation. Presented will be an integrated setup of MS, NMR and UV for routine characterization of NCEs. High accuracy MS is applied to determine the sum formula, either de-novo or as verification, while NMR provides complementary structural information. With regard to MS, the use of isotope patterns in addition to exact mass determination turned out to be essential to work in a fast, highly automated environment with high result confidence. If needed, high mass accuracy MS/MS information can be added for the verification of the proposed sum formulae.

The Metabolic Profiler™ combines a liquid handler, a 600 MHz NMR spectrometer with flow probe and a LC-ESI-(Q)TOF MS all under control of an oracle based order management system form the integrated system. The complete process with preparation and acquisition is fully automated. No manual data processing is needed. Statistical evaluation is done on the basis of PCA or other algorithms to provide relevant information for the complex sample. The data is summarized in a single comprehensive reporting system.

P127 Session 1, Thursday 1:00 - 2:30 p.m.

Highly sensitive MALDI mass spectrometry for high-throughput metabolic profiling

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(1) Kyushu University

MALDI-mass spectrometry (MS) has several advantages in metabolomics. It is a highly sensitive, high-throughput, and low sample-consuming (within 1 μL) technique compared to other analytical platforms based on LC-MS or CE-MS. In the present study, high-throughput and non-targeted metabolomic technique using MALDI-MS was developed for the rapid analysis of cellular metabolites.

Either detection limit or linearity between concentrations of several standards and peak intensities was examined, indicating the detection limit lower than 10 fmol/well and high linearity at low concentrations. To verify the validity of the method, metabolites from human leukemia cells were analyzed. Cells suspended in PBS were directly dropped onto a stainless MALDI sample plate, followed by mixing with matrix (methanol solution) on the sample plate. Only 2500 cells (0.5 μL of 5 x 10^6 cells/mL) were applied onto the sample plate. Low-molecular weight metabolites were analyzed in negative ion mode. More than 100 metabolite peaks were detected from the single analysis (200 shots/sample) within 90 seconds.

For multivariate analysis of human acute lymphoblastc leukemia Jurkat cells against drug-treatment, three anticancer drugs (methotrexate, 5-fluorouracil, and epigallocatechin-3-*O*-gallate, a green tea polyphenol) were utilized. Prior to MS analysis, an inhibitory effect of these drugs on cancerous cell growth was clearly observed. Principle component analysis on metabolites showed a clear observation of independent clusters for cells treated with these anticancer drugs. Furthermore, several metabolites involved in ribonucleotide synthesis were found to contribute to the separation of each cluster. These data suggest that the high-throughput MALDI-MS-based metabolomic technique proposed in the present study can be utilized for the drug screening and validation of drug efficacy and safety.

P128 Session 2, Thursday 4:30 - 5:30 p.m.

The good, the bad, and the ugly: NMR-based approaches for quantifying metabolites in complex solutions

Ian A. Lewis (1), Marco Tonelli (1), William M. Westler (1),John L. Markley (1)

University of Wisconsin-Madison

Over the past few years, the Madison Metabolomics Consortium has developed several approaches for identifying and quantifying individual metabolites in complex solutions. In contrast to traditional metabolomics methods, which rely heavily on multivariate statistics, our methods blend traditional analytical chemistry with two dimensional NMR spectroscopy. Although all of our methods yield molar concentrations of specific metabolites, the sensitivity, accuracy, and general utility of these strategies varies widely between techniques. We will present the pros and cons of three strategies: Fast Metabolite Quantification by ^{1}H-^{13}C NMR (FMQ by NMR), Isotope Assisted Differential Metabolomics, and Dilution Elimination TOCSY (DETOCS). The utility of these approaches will be illustrated with biological applications to metabolic flux in red blood cells and steady state analyses of osmolytes in *Arabidopsis thaliana*.

Supported by a NHGRI fellowship to I.A.L. (1T32HG002760) and by NIH grant R21 DK070297 (M.R.S., PI). NMR data were collected at the National Magnetic Resonance Facility at Madison (supported by RR02301).

P129 Session 3, Friday 4:00 - 5:30 p.m.

Quantitative profiling of bile acids in biofluids and tissues: Compound class targeting in a metabolomics workflow.

Ivana Bobeldijk (1), Suzan Wopereis (1), Maarten Hekman (1)

(1) TNO Quality of Life, Zeist

Recently we reported a sensitive, generic method for quantitative profiling of bile acids and other endogenous metabolites in small quantities of various biological fluids and tissues [1]. In this method, the best of two worlds are combined: comprehensive profiling of endogenous metabolites and fully validated quantitative determination of bile acids. The method is based on a simple sample preparation, HPLC-MS and ionisation in the in ESI-. Detection is performed in full scan using the linear ion trap Fourier transform mass spectrometer (LTQ-FTMS or LTQ-Orbitrap) generating data for many (endogenous) metabolites. We now report an improvement of the method by using UHPLC for separation. This results in significant shortening of the analysis time (from 40 minutes to 17 minutes) without sacrificing performance characteristics. A validation of the method in urine, plasma and liver was performed for 15 bile acids including their taurine, sulphate and glycine conjugates. The method was successfully applied to livers from transgenic apoE3Leiden mice that developed high fat induced insulin resistance. Although no statistically significant difference was observed in the total bile acid content (sum of bile acids measured with the LCMS method) in the high fat diet groups compared to mice on control diet, statistically significant differences were observed for several individual bile acids.

[1] I. Bobeldijk, M. Hekman, J. de Vries-van der Weij, L. Coulier, R. Ramaker, R. Kleemann, T. Kooistra, C. Rubingh, A. Freidig, E. Verheij. Quantitative profiling of bile acids in biofluids and tissues based on accurate mass high resolution LC-FT-MS: Compound class targeting in a metabolomics workflow. doi:10.1016/j.jchromb.2008.05.008

P130 Session 1, Thursday 1:00 - 2:30 p.m.

Lipidomics in studies of lifestyle associated diseases: Part 1: analytical methods.

Ivana Bobeldijk (1), Suzan Wopereis (1)

(1) TNO Quality of Life, Zeist

Changing concentrations of the different lipid classes known in plasma and tissues are important markers of life style associated diseases such as obesity, diabetes type II, liver steatosis, systemic inflammation, artherosclerosis to name just a few. (Semi)- quantitative profiling of lipids is therefore an important area in metabolomics and metabolite profiling. Differences in lipid concentrations give mechanistic insight in onset, progression and/or status of the above mentioned diseases.

There are many lipid classes present in plasma and biological tissues. They differ in their chemical properties and have a wide concentration range between classes, but also within a lipid class.

Analysis of all lipids with a single method is not possible, and therefore one has to select the best approach or analysis package depending on a particular study objective.

We/ TNO developed several lipidomics methods which can be applied individually or in combinations in order to obtain as much information as needed to obtain hypothesis confirmation or new hypothesis formulation. The common feature of all the methods is the small sample amounts and volumes required.

This poster (Part 1) will provide an overview of the LC-MS based methods comprising of NP or RP HPLC separation, direct infusion nanospray, derivatisation and various. ionization techniques. The following lipid classes are covered: TGs, ChEs, PCs, LPCs, SPMs, DGs, bile acids, fatty acids (with baseline w-3 and w-6 separation), and ceramides. All the different methods can be applied to a single lipid extract of very low sample volumes/ amounts (10 L of plasma or 5 mg lyophilized or homogenized tissue for example liver). Application of selected methods in a systems biology mice study will be presented in Part 2 of this poster, see S. Wopereis et al., Lipidomics in life style associated diseases. Part 2: biological interpretation

P131 Session 2, Thursday 4:30 - 5:30 p.m.

An improved metabolite extraction method for the yeast *Saccharomyces cerevisiae*

James Cox (1)

University of Utah

The yeast *Saccharomyces cerevisiae* is commonly used in the study of eukaryotic cellular processes. Our laboratory uses yeast to study how metals affect metabolic pathways. To perform these studies a high throughput GC-MS method for the analysis of yeast was developed. Yeast have a thick outer cell wall that prevents the simple extraction of intracellular metabolites. Previous extraction methods have relied upon a combination of chemical and physical disruption of this outer wall to extract metabolites. One frequently used method involves the addition of boiling hot ethanol (aq) to the cell pellet, followed by incubation at 85°C for five minutes. The biomass is removed by centrifugation. This method suffered from several problems in our hands. Many metabolites such as phosphorylated intermediates were destroyed or altered in this process and methods that use boiling solvents are slow not amenable to high throughput applications. A second method lyses yeast with several freeze thaw cycles after the addition of cold methanol to the cell pellet. Unfortunately, cold methanol does not completely denature metabolic enzymes and allows for continued metabolism.

Glass beads in conjunction with a vortex mixer are routinely used to extract protein from yeast. Pellets are typically suspended in an equal volume of 0.5 mm glass beads and solvent is added. Extraction takes place over eight minutes, mixing for 30 seconds followed by cooling on ice for 30 seconds, repeating for a total mixing time of 4 minutes. This method is not ideal for metabolomics due to the lengthy process and higher temperature which allows for continued metabolism.

Our laboratory modified the glass bead method by substituting the vortex for a high throughput tissue and cell homogenizer, the FastPrep-24 (MP Biomedicals). The FastPrep-24 is a high energy bead mill that can process 24 samples simultaneously. The extraction time, input energy and bead type were tested and optimized. Twenty five seconds of shaking at maximal power was sufficient to fully extract yeast cultures, decreasing sample extraction time ten-fold. Addition of a cold adaptor prevented an increase in temperature due to kinetic energy, further ensuring high quality results.

To fully arrest metabolism and simplify the chromatography, an adaptation of the Bligh-Dyer method of differential extraction was implemented. A mixture of methanol, water and chloroform is used to partition polar and non-polar metabolites between aqueous and organic phases. The solvent mixture was added to the extraction tube prior to homogenization. The solvent system had several advantages: it quickly denatured metabolic enzymes, removed much of the non-polar metabolites that interfered with chromatography, and improved the yield of phosphorylated metabolites.

A high throughput method of metabolite extraction from yeast was developed. This method decreased the time needed for sample preparation, improved the data quality and increased the yield of unstable metabolites.

P132 Session 3, Friday 4:00 - 5:30 p.m.

Global metabolic profiling using LC-FTMS based upon reproducible m/z features

Jennifer M. Johnson, Tianwei Yu, Frederick H. Strobel, Dean P. Jones

(1) Emory University

Metabolic profiling provides an overview of the metabolic status and global biochemical events with the promise to enable more accurate assessment of dietary deficiencies, detection of disease states and their progression, monitor response to therapy and exposure to environmental toxins. Initial studies using NMR, GC-MS and LC-MS have found that anywhere between 50-90% of the small molecules detected (depending on the method) do not correspond to known metabolites and the utility of global metabolic profiling has been questioned. In the current study, we designed a method for global metabolic profiling that is based on a short chromatographic separation followed by accurate mass detection in a Fourier Transform mass spectrometer (FTMS) to test the feasibility of using the method in global metabolic profiling. Plasma was collected from 4 healthy free-living volunteers and was run 8 times on each of 2 columns to test intra-sample, inter-sample and inter-column variability. Peak detection was done using an in-house developed software package with accurate mass, retention time and peak shape as criteria. Results show a total of 1995 features are detected with 22% returning in a search using the Madison Metabolomics Consortium Database (MMCD). Analysis of run-to-run variability showed that 85% of features were detected in consecutive runs, and more than 80% of the features had a coefficient of variation (CV) less than 10%. 1669 features were present in at least 50% of spectra. 1014 were present in all individuals. 179 features were unique to 1 individual probably due to dietary and genetic differences. Principle component analysis (PCA) showed that inter-individual variation was greater than intra-individual variation and statistical analysis of microarrays (SAM) analysis using a conservative false discovery rate (FDR) of 0.1% found 488 features that explained the inter-individual variation. Of these 24% correspond to known chemicals in the MMCD. 3D landscape projections of these 488 features showed each individual's unique metabolic fingerprint. These results indicate that global metabolic profiling based upon reproducible m/z features can be used in the creation of a cumulative metabolic library for use in clinical settings.

P133 Session 1, Thursday 1:00 - 2:30 p.m.

metaP: Introducing a high-throughput metabolomics platform at the Helmholtz Zentrum

Cornelia Prehn (1), Werner Romisch-Margl (2), Philippe Schmitt-Kopplin (3), Karsten Suhre (2), Jerzy Adamski (1)

(1) German Research Centre for Environmental Health, Institute of Experimental Genetics (2) German Research Centre for Environmental Health, Institute of Bioinformatics and Systems Biology (3) German Research Centre for Environmental Health, Institute of Ecological Chemistry

MünchenMetabolomics is a very fast expanding research field for phenotyping of biological samples with an unbiased approach of characterisation. Especially, either not much pronounced (silent) phenotypes or subsidiary phenotypes could be determined if many different parameters are correlated. At present, two main approaches in metabolomics are performed: targeted (quantification of a chosen set of metabolites) and non targeted (profiling or search for biomarkers).

The Metabolomics Platform (metaP) of the Helmholtz Zentrum München is designed to mediate progress in science through development of new methods for metabolomics and provision of measurement services applicable to human, animal models, plants, environmental samples and ex vivo systems. Part of our activities is related to targeted metabolomics, where high-throughput quantification based on the Biocrates Absolute IDQ Kit technology covers more than 150 endogenous metabolites like lipids, amino acids, acylcarnitines, carbohydrates and many more. To achieve these results, a sample amount of only 10µl plasma is needed. Stringent tests with a high number of replications in different sample types show that the measurements perform very well and with high reproducibility. We successfully conducted studies in the population based human KORA (Cooperative Health Research in the Region of Augsburg) cohort and in animal models in elucidating metabolomic effects in complex diseases or drug development, respectively. To facilitate high quality standards and sample tracking we built up a tailor made LIMS. The non-targeted approaches are followed up on in a lower throughput manner, using two-dimensional chromatography coupled to mass spectrometry and flow injection ultrahigh resolution mass spectrometry with various ionization modes. To support this task, we recently established the MassTRIX web-service (http://masstrix.org) for annotating metabolite elemental compositions from ultrahigh resolution mass spectrometry data in their genomic context on KEGG pathway maps. The processivity of the Metabolomic Platform from HTP screening to structure identifications is reached by integrating different expertise on the campus of the Helmholtz Zentrum München. At present, in the metaP consortium the following groups collaborate: robotics for sample preparation and analyte quantification (LC-MS/MS 4000 QTrap; Jerzy Adamski), high resolution analysis and profiling (12 Tesla FTICR-MS; Philippe Schmitt-Kopplin), biobanking (Thomas Illig) and bioinformatics/project integration (Karsten Suhre).

Altmaier E, Ramsay SL, Graber A, Mewes HW, Weinberger KM, Suhre K. Bioinformatics analysis of targeted metabolomics-uncovering old and new tales of diabetic mice under medication. Endocrinology. 2008 Jul;149(7):3478-89 Chen, J., X. Zhao, R. Lehmann, J. Fritsche, P. Yin, Ph. Schmitt-Kopplin, W. Wang, X. Lu, H.U. Häring, E. D. Schleicher, G. Xu, Strategy for biomarker discovery and identification based on LC-MSn in metabonomics research., Anal. Chem., (2008), 80, 1280-89

P134 Session 2, Thursday 4:30 - 5:30 p.m.

Detection of acetaminophen urinary metabolites using liquid chromatography/time of flight mass spectrometry and multivariate analysis

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(1) NCTR/USFDA

Understanding of a drug's metabolic fate is extremely important for drug discovery and development. Metabonomics based on mass spectrometry with exact mass accuracy combined with multivariate statistical principal component analysis (PCA) can provide detection of drug metabolites rapidly lowering both the cost and time associated with the drug discovery process. The strategy described here using acetaminophen (APAP) as a model compound showed the capability to detect APAP metabolites in urine samples that result from predictable and unpredictable metabolic transformations. The PCA scores plot shows a clear separation in groups 8 hrs post-dosing with vehicle (control), acute administration (rats treated with acute single dose of 400 mg/kg or 1600 mg/kg APAP) and chronic dosing (rats dosed daily with 200, 400 or 800 mg/kg APAP). Using this approach, not only were the major (APAP, APAP-sulfate, APAP-glucuroninide, APAP-N-acetyl-L-cysteine (APAP-NAC)) and minor metabolites (APAP-cysteine, APAP-$SOCH_3$ and APAP-S-S-APAP) of APAP detected but also some APAP-related ions from unpredicted metabolic pathways were successfully detected and even identified. Detection and identification of drug metabolites in urine can be used to predict drug safety and drug efficacy. After an APAP overdose, glutathione (GSH) can deactivate N-acetyl-p-benzoquinone imine (NAPQI, reactive intermediate responsible for tissue damage) to form an APAP conjugate (APAP-GSH). The corresponding conjugates including APAP-NAC, APAP-cys, APAP-$SOCH_3$ and APAP-S-S-APAP are breakdown products of APAP-GSH. Results show that APAP eliminated in urine as APAP-NAC was extremely low in the animals dosed with sub-toxic concentrations of APAP, but was extremely high in the group administered a toxic dose on day 2, which is consistent with histopathology data that showed tissue damage only in the high dosed animals on day 2. Changes in the composition of drug metabolites can also be used to deduce alterations in the endogenous metabolite due to the connectivity between a drug, drug metabolites, and endogenous metabolism. Large amounts of APAP-NAC were present in urine from both low dosed and high dosed animals on day 1, while on day 2 only urine samples from high dosed rats had a larger concentration of APAP-NAC. Results were consistent with the decreased concentration of S-adenosylmethionine (SAMe, primary sulfur source in the biosynthesis of glutathione) in urine for both the low dosed and high dosed animals on day 1 and the high dosed group on day 2. This approach should be of importance for the detection of drug candidate metabolites in biofluids with a rapid speed during drug screening development.

P135 Session 3, Friday 4:00 - 5:30 p.m.

Small Molecule Metabolite Identification in Complex Biological Samples Using Comprehensive Two-Dimensional Chromatography Combined with Time of Flight Mass Spectrometry (GCxGC-TOFMS)

John Heim and Mark Libardoni

LECO Corporation

Small molecule metabolite analysis presents challenges that historically have relied heavily upon standard quadropole GC/MS utilizing targeted methods of selected ion monitoring and tandem GC/MS/MS mass spectrometric techniques. The complex nature of metabolomic samples demands

analytical solutions and instrumental methods that will identify the small molecule metabolomic profile completely as well as discover significant key components of interest. Comprehensive two-dimensional gas chromatography (GCxGC) expands the peak capacity of the chromatographic separation thereby increasing resolution and analyte characterization necessary for complex biological samples. Two orthogonal separation phases (such as nonpolar and polar) are utilized to maximize separation capacity in a single analysis. The high data density and narrow peak widths inherent to GCxGC analysis requires a detection system able to characterize the peak shape and small molecule metabolite identification. Time of flight mass spectrometry (TOFMS) offers continuous full range nonskewed mass spectral information and fast acquisition rates ideal for metabolomic identifications. The combination of TOFMS data and deconvolution algorithms facilitates trace level analyte detection that would otherwise be hidden and coeluted with other compounds in the sample. This presentation will show metabolomic data that illustrates the benefits of multidimensional chromatography coupled with time of flight mass spectrometry. Two dimensional chromatographic plots of biological samples showing increased peak capacity and structural orientation not possible in one dimensional chromatography will be highlighted. The metabolomic sample data presented will show increased analyte detectability as a result of cryo-focusing in the GCxGC process. In addition, classifications for specific chemical functional groups can be utilized as a user defined data mining method to aid in data reduction and increase overall experimental results. Sample groups representing normal and disease state will also be discussed.

P136 Session 1, Thursday 1:00 - 2:30 p.m.

Analysis of Lipids by TAP Fragmentation using a Hybrid Quadrupole/Travelling Wave Ion Mobility/oa-TOF Mass Spectrometer

John Shockcor

University of Cambridge, Waters Corp.

Ion mobility mass spectrometry (IMS) allows separation of ionic species as they drift through a gas phase under the influence of an electric field. The rate of an ions drift depends on the mass of the ion, its particular charge state and the average cross-sectional of the ion. It is possible to separate ions with the same nominal mass if they have different charge states or different interaction cross-sections.

In the hybrid quadrupole/Travelling Wave IM/oa-TOF instrument, the instrument was configured with three traveling-wave (T-wave) devices in sequence. The second T-wave functions as an ion mobility drift tube, the pre-IMS T-wave traps ions, and the post-IMS T-wave transfers the ions after they are separated from drift tube. An interesting consequence of this configuration is that the pre-IMS T-wave and post-IMS T-wave can function as two separate collision cells and produce fragment ions independently. The fragment ions produced in the trap T-wave (pre-IMS) can be separated based on their charge states and their size as they move through the IMS. These ions separated by their different drift times can then be further fragmented in the transfer T-wave (post-IMS). As a result, the fragment ions generated in transfer T-wave are drift time aligned with their respective precursor ions resulting in a Time Aligned Parallel fragmentation (TAP) pattern. If a single precursor ion is selected in the quadrupole stage prior to the Tri-Wave, this TAP fragmentation pattern is in effect offering some very selective MS3 information for the compound of interest.

In this presentation we have chosen to illustrate these concepts by evaluating the ion-mobility characteristics and applicability of the TAP fragmentation to the study of lipids (lipidomics). Molecules from different lipid classes as well as extracted rat plasma lipids were examined for this purpose. The TAP fragmentation patterns of these compounds were analyzed with a chemically-

intelligent software tool providing a rapid confirmation of the proposed structures. In addition, the reproducibility of the drift times of these compounds were studied during the course of the experiments.

P137 Session 2, Thursday 4:30 - 5:30 p.m.

Improving the odds of finding needles in haystacks: Metabolomics by diffusion NMR

Jonathan Byrne (1), Clare Daykin (1), Florian Wulfert (1), Rasmus Bro (2)

(1) University of Nottingham (2) University of Copenhagen

To our knowledge, all published data in the field of metabolomics investigates changes in either absolute or relative quantities of metabolites. However, blood plasma, one of the most commonly studied biofluids for metabolomics applications, is a complex, heterogeneous mixture of lipoproteins, proteins, small organic molecules and ions which together undergo a variety of possible molecular interactions including metal complexation, chemical exchange processes, micellular compartmentation of metabolites, enzyme-mediated biotransformations and small-molecule-macromolecule binding. In particular, many low molecular weight (MW) compounds (including drugs) can exist both 'free' in solution and bound to proteins or within organised aggregates of macromolecules. To study the effects of e.g. disease on these interactions we propose the application of a technique we have termed 'interactive metabolomics'. Interactive metabolomics can be defined as: "*the non-selective study of interactions between low MW biochemials and macromolecules in heterogenous biosamples such as blood plasma, without pre-selection of the components of interest*".

The standard one dimensional (1D) NMR experiment commonly applied in NMR-based metabolomics allows the study of metabolite concentration differences between groups of samples because the intensity of each peak is dependent upon the concentration of the compound in question. On the other hand, the instrument can be set-up to measure molecular interactions by monitoring the diffusion coefficients of molecules. According to the Stokes-Einstein equation, the diffusion coefficient of a molecule is directly proportional to its hydrodynamic radius. Therefore, when low molecular weight compounds are non-covalently bound to proteins, the observed diffusion coefficient for the compound will be a weighted average of its free and bound forms. By measuring diffusion by NMR, an estimation of the degree of protein-binding can be made for either low molecular weight endogenous biochemicals or xenobiotics. This type of experiment is commonly referred to as either Diffusion-Ordered Spectroscopy (DOSY) or Diffusion-Edited Spectroscopy, depending on the type of post-acquisition data processing which is applied to the spectra.

The post-acquisition data processing and analysis of diffusion-NMR data is crucially important for extraction of accurate diffusion coefficients, must be efficient, reliable and give accurate and reproducible results. As part of a wider project to apply high-throughput diffusion NMR to metabolomics, methods for pre-processing and analysis of PFG-NMR data have been tested. The data used for this testing were obtained from; computer simulations; simple multi-component mixtures of small molecules; samples of small molecule metabolites at varying concentrations in the presence of macromolecules (bovine serum albumin); and human plasma samples. The PARAFAC algorithm[3] was of particular interest, as it yields pure components and pure decay profiles.

The results of the study show the enormous potential of the approach for non-selectively monitoring changes in the interactions of metabolites in solution. With this aspect of the data flow optimized, great advances have been made towards applying the extremely powerful diffusion-NMR method to

important biological problems. The metaphorical needles (those metabolites exhibiting differences in diffusion coefficients between samples) should be more obvious in their respective haystacks (1D ^{1}H NMR spectra) by virtue of differences in their curvature, as determined by PARAFAC.

[3]R.Bro, "PARAFAC: Tutorial and applications", Chemom.Intell.Lab.Syst., 1997, 38, 149-171.

P138 Session 3, Friday 4:00 - 5:30 p.m.

Ion Mobility Spectrometry for Metabolic Profiling of Human Breath

Jörg Ingo Baumbach (1), Wolfgang Vautz (1), Alexander Bunkowski (1), Bertram Bödeker (1), Michael Westhoff (2)

(1) ISAS - Institute for Analytical Sciences, Metabolomics Department (2) Lung Hospital Hemer

It is well known, that human breath acts as carrier of information on the metabolic processes in the human body. Because of the fact, that early diagnosis of lung cancer and airway infections is gaining increasing importance, we have examined whether volatile metabolites in human exhaled air can be correlated directly to different kinds of diseases using ion mobility spectrometry. The IMS investigations are based on different drift times of swarms of ions formed from the metabolites directly in air at ambient pressure. About 10 mL of breath is necessary to carry out a full analysis.

For investigations of human breath at a high level of humidity a combination of a Multi-Capillary Column (MCC) partly pre-separating the analytes is used in combination with a conventional ion mobility spectrometer (IMS). An IMS coupled to a MCC allows for the identification and quantification of volatile metabolites occurring in human breath down to the ng/L- and pg/L-range of analytes within less than 500 s and without any pre-concentration steps directly.

The full procedure, including sampling, pre-separation and identification of metabolites in human exhaled air, was developed and implemented with a view to future use in hospitals. The talk will discuss fundamentals of the analytical method ion mobility spectrometry and present successful applications. Examples of proven trials considering lung cancer, COPD (chronic obstructive pulmonary disease) and sarcoidosis will be presented.

P139 Session 1, Thursday 1:00 - 2:30 p.m.

Identification of metabolomic components from urine using an accurate mass retention time (AMRT) database and a molecular formula generator

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(1) Agilent Technologies Inc., Santa Clara, CA, USA

Untargeted metabolomics workflows typically include sample preparation, data collection, feature finding, statistical analysis and compound identification. A significant challenge for compound identification is the absence of a comprehensive LC/MS based database of retention time matched compounds. In collaboration with the Scripps Research Institute, over 25,000 mass entries in the METLIN database have added to create a custom database which includes the ability to add Retention time (RT) and possesses an automated Molecular Formula Generation (MFG) capability that uses chemical rules, including isotopic pattern matching to enable higher confidence matching of compounds from accurate mass data. The software has the capability of creating custom

databases, to which new compounds can be added. To demonstrate the utility of this customizable personal metabolite database, a library of urine metabolites was developed from a limited set of 78 standards, representing some of the compounds normally found in urine. Human urine samples were then analyzed by LC/ESI- time-of-flight (TOF) mass spectrometry (MS) and screened for mass and RT matches against this database to generate a list of masses with a specific RT, a metabolite compound match and a putative formula(s). MFG provided additional confirmation of the compound identification generated by the database search.

Identified metabolites were annotated with a chemical formula, structure and other metadata, including CAS and KEGG identifiers. A total of 1070 compounds were found to be present. From these, 436 had a match to the database and only 4 did not correspond to the expected molecule formulae. This is a very high success rate for this approach of metabolite identification. For masses that do not have a database match the molecular formula generated can still be used to guide further experiments for compound identification and in this case 563 compounds were not matched but each did generate a suggested molecular formula. If identification was to be made on these compounds then there is capability to include this new information in a custom database.

P140 Session 2, Thursday 4:30 - 5:30 p.m.

Influences of sample collection and handling procedures of biofluids on metabolomics studies.

Kiyoko Bando(1), Rui Kawahara (1), Emiko Saijo (2), Takeshi Kunimatsu (2), Juki Kimura (2)

(1) Osaka University (2) Dainippon Sumitomo Pharma.Co.Ltd.

Metabolomics is increasingly being applied to pharmacology and toxicology studies. In many cases, animal experiments are designed, and biofluid (urine, plasma, et al.) samples are collected from animals and analyzed. An important requirement in animal-based pharmacology or toxicology is minimization of variation in control animals relative to the changes induced by drugs. In addition to genetic, physiological, and environmental factors that result in variation of biological status in metabolomics studies, technical factors such as sample collection and storage should also be addressed. We wish to present an investigation of the influence of sample collection and handling procedures such as method of collection and storage temperature on the metabolic profiling.

In this study, we examined several collection and handling procedures of urine and plasma samples from male rats as follows: Urine: Time of pooling urine (4 or 24 hours), temperature during collection (room temperature (R.T.) or frozen. Plasma: conditions of animals (fasting, anesthesia, the site of blood sampling), anticoagulants (EDTA-2K or sodium heparin) for preparing plasma.

Samples were analyzed using a gas chromatography-time-of-flight-mass spectrometer (GC-TOFMS, Agilent Technologies Leco) and an ultra performance liquid chromatography-time-of-flight-mass spectrometer (UPLC-TOFMS, Waters), and data were treated with software tools for peak finding/alignment algorithms (ChromaTOF, Line-up for GC-TOFMS, MZ-mine for UPLC-TOFMS). Next, the generated peak lists were imported into SIMCA-P+ (Ver.11.0) for multivariate statistical analysis.

As a result of urine analysis using GC-TOFMS following PCA, the score plot showed a clear clustering of the two groups: 4-hr pool and 24-hr pool, and spots of 4 hr-pooled samples were much more scattered than those of 24hr-pooled samples. In addition, spots of iced samples were less scattered than those of R.T. samples. In the meantime,, PCA of the resulting data using UPLC-TOFMS also clearly indicated the clustering of the 4-hr and 24-hr pools, but there was no difference in dispersion of spots between the two groups, and then temperature (R.T or frozen) did not contribute to clear clustering. For plasma, PCA score plot showed the separation of groups depending on the sampling

procedures, but the dispersion of spots of each sample was similar among groups except for data derived from plasma treated with sodium heparin using GC-TOFMS.

This study showed that we might have different results depending on sampling and handling procedures in animal experiments. Especially, short time collection of urine is not suitable because it may emphasize individual variation, and appropriate anticoagulant should be chosen for preparing plasma depending on analytical equipment. In any event, it is considered that a dataset should not be compared to another dataset derived from samples by a different method. We will present in more detail the results of our investigation on factors which might affect data and interpretation of biological status.

P141 Session 3, Friday 4:00 - 5:30 p.m.

Plasma lipid profiling using chip-based direct infusion nanospray tandem mass spectrometry

Kwang-Hyeon Liu (1), Do Yup Lee (1), Tobias Kind (1), Oliver Fiehn (1)

(1) UC Davis

We present a comparison of different solvent extraction systems for profiling polar lipids using an automatic workflow based on robotic nanoelectrospray-ion trap tandem mass spectrometry. Infusion times were two minutes per sample from 300-900 m/z with positive ionization. Spectra were aligned by the Genedata Expressionist Refiner MS software directly from raw files. The workflow includes grid alignment of spectra, spectral averaging, cutting out low intensity signals, detection peaks, modeling peak shapes and eventually, exporting the alignment results. This post-processing took around 15 seconds for 50 samples. Additionally, data dependent MS/MS spectra were extracted in the Refiner MS software which were performed on the 30 highest peaks in the full scan spectrum and scanned in low resolution mode with 20V CID voltage. A matrix containing all samples and all aligned ions above a certain signal noise threshold was obtained as a result file. Lipid species were annotated by mass and MS/MS lookup tables that were constructed from the LipidMaps database for which we added 64,000 potential lipids species such as lipids with short-chain or odd fatty acyl chains. We then performed a comparison of five representative extraction protocols such as protein precipitation method using isopropanol/acetonitrile/water (Fiehn method) or acetone/methanol (Agilent method) as extraction solvent and liquid-liquid extraction method using chloroform (Forch and Bligh & Dyer method) or tert-butyl methyl ether (Matyash method) as extraction solvent for their ability to extract lipids from plasma samples. Quantitative readouts were evaluated using unsupervised and supervised statistical analyses. While all extraction protocols provided precision within a median of at least <23% CV over all detected lipids, it was also evident that each extraction method resulted in distinct clusters in unsupervised PCA plots. This finding suggests that there is a clear influence of the solvent composition and method details on the recovery of lipid extractions. The highest abundance of lipid ions and good precision of 17.1% CV was found for the Matyash method (J Lipid Res, 2008, 49: 1137-46) which uses tert-butyl methyl ether as extraction solvent.

P142 Session 1, Thursday 1:00 - 2:30 p.m.

Microbial metabolomics: Toward a platform with full metabolome coverage

Leon Coulier

TNO Quality of Life

Achieving metabolome data with satisfactory coverage is a formidable challenge in metabolomics because metabolites are a chemically highly diverse group of compounds. Here we present a strategy for the development of an advanced analytical platform that allows the comprehensive analysis of microbial metabolomes. Our approach started with in silico metabolome information from three microorganisms— Escherichia coli, Bacillus subtilis, and Saccharomyces cerevisiae—and resulted in a list of 905 different metabolites. Subsequently, these metabolites were classified based on their physicochemical properties, followed by the development of complementary gas chromatography–mass spectrometry and liquid chromatography–mass spectrometry methods, each of which analyzes different metabolite classes. This metabolomics platform, consisting of six different analytical methods, was applied for the analysis of the metabolitesfor which commercial standards could be purchased (399 compounds). Of these 399 metabolites, 380 could be analyzed with the platform. To demonstrate the potential of this metabolomics platform, we report on its application to the analysis of the metabolome composition of mid-logarithmic E. coli cells grown on a mineral salts medium using glucose as the carbon source. Of the 431 peaks detected, 235 (=176 unique metabolites) could be identified. These include 61 metabolites that were not previously identified or annotated in existing E. coli databases.

P143 Session 2, Thursday 4:30 - 5:30 p.m.

High-throughput non-targeted metabolic profiling with hybrid stationary phase LC column coupled to Q-TOF-MS in cervical hyperplasia

Hyun-Jin Jung (1), Man Ho Choi (1), Yong-Il Kwon (2), Won-Yong Lee (3), Bong Chul Chung (1)

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In contrast targeted analysis, non-targeted metabolic profiling involves a large number of different metabolites with the objective of identifying a specific metabolite responsible for biological changes. A rapid and reproducible sample preparation is necessary in non-targeted metabolomics because it may affect reproducibility as a result of the heterogeneity of metabolites derived from cell populations. Here, we introduce a non-targeted metabolic profiling technique using hybrid stationary phase LC column coupled to a quadrupole-time-of-flight mass spectrometer (Q-TOF-MS) to identify altered metabolites and to find potential biomarkers which may indicate progress of cervical displasia. A column switching system constructed for the HPLC system coupled to a 6-port 2-way valve and a 200 μL urine filtered with Ultrafree-MC Durapore (PVDF, 0.1 μm × 0.5 mL) was directly injected into a hybrid column (Cadenza HS-C18, 2.0 mm i.d. × 100 mm, 3 μm particle size) at a flow-rate of 400 μL/min to isolate endogenous interferences. After 3.0 min, a switching valve was turned to the alternate positions to elute metabolites with a gradient mobile phase to complete separation and MS detection within 14 min-running time. Both retention time and spectral data for each chromatographic peak were paired and normalized with the ion intensities in data processing. All data processed were statistically analyzed by partial least-squares discriminant analysis (PLS-DA) and hierarchical clustering analysis (HCA). The present non-targeted metabolic profiling was applied to urine samples obtained from 21 patients with cervical dysplasia and 21 healthy controls.

According to PLS-DA and HCA, 39 discriminating ions as the potential biomarkers were detected and most of them were found in relatively higher concentrations in patients compared to controls because malignant tumors have higher metabolic turnover rates. Especially, large peaks eluted at 4.06 and 7.65 min were only detected in patients and these peaks have m/z 132, 227, 240 and 326 (at 4.06 min) and m/z 179, 277, 319, 337, 377, 393, 579 (at 7.65 min). The human metabolome database was used to identify 2 potential biomarkers produced and they might be correlated with lipid or energy metabolism.

P144 Session 3, Friday 4:00 - 5:30 p.m.

High resolution spectroscopy for metabotyping of mutants from Drosophila melanogaster

Mhmd Anas Kamleh (1), Yahya Hobani (2), Julian Dow (2), John Parkinson (1), David.G Watson (1)

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Zwitterion hydrophilic interaction chromatographic system coupled to a high resolution Fourier Transformation Mass Spectrometre (LTQ Orbitrap®) was used to outline the metabotype of different genotypes of Drosophila melanogaster. The four genotpypes studied were Wild type (Canton S), and the three eye-colour mutants rosy, maroon-like and Chocolate. Multivariate analysis was used to observe how similar the MS profile was for the four genotypes, spot outliers as well as to extract what masses are more likely to be of high contribution to the model obtained. Because of the busy nature of MS loadings plot, statistical correlation was used to broaden the view and further discern important variables.

The method has shown HILIC chromatography to be of great value for separating polar metabolites like amino acids and phosphate amines. The rosy mutation is the second oldest naturally occurring mutation, lacking the enzyme xanthine oxidase which converts hypoxanthine to xanthine and subsequently xanthine into uric acid. The biosynthesis of uric acid is of high importance for the metazoans as it is the most suitable route for nitrogen excretion. The lack of ability to make urate (and the corresponding accumulation of xanthine) in rosy flies makes the flies weaker, and they only survive on a low purine diet. It was long thought that rosy`s metabolome was altered in the purine synthesis pathway. Our study has confirmed this to be a major consequence of the mutation. However, mass spectrometry has implicated other pathways, independent of purine metabolism. Glycerphosphocholine (GPC) and glycerphosphoethanolamine (GPE) were both shown to be remarkably elevated in rosy compared to wild type. The rosy flies are unable to excrete nitrogen in the form of urate, and obliged to use xanthine for this purpose. Xanthine is more water soluble than urate, forcing the flies to a parallel loss of more water. GPC and GPE are known to play a counter osmolyte rule as a response of the high osmolality. It was found that 3-hydroxykynurenine levels were lower in and kynurenine was correspondingly elevated, suggesting that the enzyme responsible for 3-hydroxylating kynurenine displays reduced activity thus affecting ommochrome pigments which are formed in Drosophila. Arginine metabolism was found to be affected as well. The effect may be a consequence of the accumulation of dihydrobiopterin (DHBT) which leads to the inhibition of tetrahydrobiopterin (THBT) recycling . THBT is a co-factor required for arginine oxidation to citrulline and NO. High resolution nuclear magnetic resonance (NMR) was also used to compare the metabotype of the four genotypes of Drosophila. Being a totally different technique, with a totally different output , NMR was used to confirm the MS findings and expand the picture into metabolites not captured by the first technique. The maroon-like (mal) mutants are also deficient in xanthine oxidase. Mal flies were compared to wt using both techniques (MS, NMR) which showed the flies to have –as expected- a profile similar to that of rosy in most of the

metabolic pathways, but different in others such as glutamine , methionine and methionine-S-oxide which were all upregulated in this genotype.

P145 Session 1, Thursday 1:00 - 2:30 p.m.

Molecular Formula dereplication based on MSn

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Identification of metabolites is essential for their use as biomarkers, for research in systems biology and for drug discovery. The first step before any structure can be elucidated is to resolve the elemental composition (EC) of the compounds with an unknown identity. High resolution mass spectrometry and common constraint rules cannot always provide one unique EC solution. Automatic multi-stage mass spectrometry (MSn) analysis is a new challenge for the dereplication of the correct EC.

Interpretation of MSn data occurs largely manually and there are almost no tools available to handle this information. Automation of the interpretation of these data will accelerate the processing mechanism and posteriorly conduct an in-depth study of the results.

This poster presents an overview of the Multi-stage Molecular Formula (MMF) method/tool which has the ability to resolve the elemental composition of the compound and fragment ions derived from MSn data using a cyclic constraining process. The method reduces for each fragment ion the list of EC's based on EC's of its fragments and parents. This procedure is repeated in a cyclical way several times until a unique EC is obtained or the list of EC candidates doesn't change anymore. Different metabolites were tested, using the MMF method, to study the necessary accuracy to resolve the elemental composition. In addition, the efficacy to detect false peaks in the spectrum will be presented.

P146 Session 2, Thursday 4:30 - 5:30 p.m.

Chemical Derivatization Approaches for Sensitivity and Resolution Enhanced Detection of ^{13}C, ^{15}N and ^{31}P in NMR Based Metabolomics

Narasimhamurthy Shanaiah (1) , Tao Ye (1) , Aruni DeSilva (1), Naganagowda Gowda (1), Daniel Raftery (1)

(1) Purdue University

^{1}H NMR spectroscopy has been widely used in body fluid analysis owing to its simplicity, quantitative and unbiased detection of proton containing metabolites, and its reasonable sensitivity. NMR spectra of untreated samples represent an overview of all metabolites present in a body fluid and can be used as a finger print, while statistical methods can be employed for biomarker discovery and validation. Despite the high utility of NMR spectroscopy in metabolomics, a continuing challenge is the need to resolve up to thousands of highly overlapped NMR resonances in biofluids.

A promising approach for improving the resolution is to observe the other NMR-active nuclei including C, N, and P that have large chemical shift ranges. Although ^{31}P have a favorable natural abundance and good relative sensitivity, there are few phosphorus containing metabolites. On the contrary, ^{13}C, ^{15}N are present in many metabolites, but suffer from their low inherent sensitivity and natural abundance, but otherwise provide a wealth of additional information on metabolites. While standard methods of observing ^{13}C or ^{15}N by NMR is still very challenging, we have developed methods to label different classes of metabolites of interest isotopically by chemical derivatization. This approach enhances sensitivity especially when combined with 2D HSQC (Heteronuclear Single Quantum Coherence) experiments. The result is a much improved sensitivity and resolution over the standard 1D carbon-detected experiment, and comparable sensitivity and improved resolution compared to ^{1}H experiments.We present here simple and robust chemical derivatization methods for introducing ^{13}C and ^{15}N reagents as isotopic labels or tags of certain classes of metabolites. The reactions are facile, occur in aqueous solution, and allow improved detection of amino acids, amines, and carboxylic acids at low concentration and in complex mixtures such as urine and serum. We have also extended this derivatization approach to label lipids containing labile hydrogens in human serum with 2-chloro-4,4,5,5-tetramethyldioxaphospholane for detection by ^{31}P NMR spectroscopy. Application of this approach to enhance the detection of metabolites in biofluids provides a convenient and sensitive method for the qualitative and quantitative analysis of various classes of metabolites that may have high utility for diagnosis and for the study of pathophysiology in various diseases.

P147 Session 3, Friday 4:00 - 5:30 p.m.

Ratiometric analysis of glutathione metabolism by Capillary Eelectrophoresis-Mass Spectrometry: an elusive clinical biomarker of oxidative stress

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Glutathione metabolism play important roles regulating antioxidant defense, xenobiotic detoxification and protein activity that is critical to overall cell function. The ratio of reduced to oxidized glutathione (GSH:GSSG) represents a widely reported yet elusive clinical biomarker of oxidative stress that has been associated with several classes of chronic disorders. Reliable methods for ratiometric glutathione analysis are hampered by the lability and high concentration levels of GSH that results in significant bias due artifactal oxidation during sample handling. In this report, a robust method for ratiomertic glutathione analysis in red blood cell lysates was developed using capillary electrophoresis-mass spectrometry (CE-MS) in conjunction with fingerprick microsampling. In this work, global assessment of antioxidant capacity was demonstrated by comparing the apparent kinetics of GSH oxidation between normal and GSH-depleted cells. CE-MS provides a sensitive, selective and reliable method to quantify glutathione metabolites over 1000-fold dynamic range without interferences.

P148 Session 1, Thursday 1:00 - 2:30 p.m.

Discerning isomeric metabolites using high resolution fragmentation trees

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Structural characterisation and identification of metabolites can in principle be achieved using high resolution multistage mass spectrometry (MSn). However, neither general methodology for the identification nor extensive databases of metabolites with multistage mass spectrometric data are available at the moment. A challenge is the lack of software and standardization for handling MSn data in order to use the full information derived from fragmentation trees, currently there is only one commercial software offering some support in this (Mass Frontier, HighChem). MSn fragmentation spectra and hierarchical dependencies between ions within fragmentation trees reflect the structural arrangement of the chemical building blocks (sub-structures, functional groups) of metabolites. We explore in this poster the feasibility of a strategy for metabolite identification based on analysis of fragmentation trees.

Data dependent acquisition of high resolution fragmentation trees of metabolites was performed on a nano electrospray-LTQ-Orbitrap system (Thermo) equipped with an automated electrospray infusion device (TriVersa NanoMate, Advion). Fragmentation trees were processed and the fragments were annotated with their elemental compositions using software and algorithm developed in our group.

Repeatability, reproducibility and robustness of fragmentation tree acquisitions were tested by changing experimental conditions and varying the concentration of the metabolite of interest. An acquisition protocol was established for the reliable and reproducible acquisition of mass spectral trees. It was investigated to which extent the variation of conditions such as fragmentation energy, isolation width etc. did change the fragmentation pattern or topology of hierarchical relations between fragments.

The high resolving power and mass accuracy of the Orbitrap detector, combined with additional information from fragmentation allowed the unambiguous annotation of observed m/z values with their elemental composition at all MSn levels (up to MS5), and thus provided much insight into the fragmentation pathways of the metabolite in the MS. The fragmentation pattern being a fingerprint of the structure of fragmented ion can then be used to distinguish metabolites with similar structure from each other. Actually, we demonstrate in the poster how the developed analytical strategy based on mass fragmentation trees can be used to discriminate between metabolite isomers with the same elemental composition and an only slightly different structure, but with a significantly different biological function.

P149 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolite profiling of perfused and non perfused rat liver tissues using accurate mass LCMS

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(1) Bruker Daltonics (2) Bruker Biospin and Pfizer Inc. (3) Bruker Biospin (4) Pfizer Inc.

Metabolite profiling of biological fluids such as blood, urine, serum are routinely performed for identifying the response to stimuli in an organism. Organs such as liver are involved in various metabolic functions including detoxification. Hence profiling tissues help understanding the metabolic pathways of interest. NMR and Mass Spectrometry are widely used techniques for performing metabolic profiling. The blood in tissues may interfere in identifying its 'true' metabolite profile. Perfusion is usually conducted on the anesthetized animal with saline solution to remove blood. This stress condition may influence the metabolite distribution in the tissue. The tissue samples were homogenized and extracted with water/acetonitrile solvent system. The separation of metabolites is carried out by liquid chromatography. We have used LC coupled ESI-QTOF mass spectrometry to probe the changes happening due to perfusion.

Principle component analysis (PCA) is a statistical tool used to analyze such complex LCMS datasets and reveals the distribution and characteristics of the compounds detected. Variant compounds are then identified using accurate mass and isotopic pattern information.

P150 Session 3, Friday 4:00 - 5:30 p.m.

Quantitative Analysis of Metabolites in Complex Biological Samples Using Ion Pair Reversed Phase Liquid Chromatography Isotope Dilution Tandem Mass Spectrometry

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(1) Delft University of Technology (2) DSM Anti-Infectives

Quantitative data on intracellular metabolite concentrations in microorganisms play an increasingly important role in metabolic engineering studies. These data have been used to understand the in vivo reaction kinetics [1, 2] for strain improvements or increased productivity in fermentation processes. Here, we demonstrate a rapid, sensitive and selective ion pair reversed phase liquid chromatography electrospray ionization isotope dilution tandem mass spectrometry (IP-LC-ESI-ID-MS/MS) for quantitative analysis of free intracellular metabolites in cell cultures. As an application this method has been developed and validated for a group of important compounds involved in penicillin biosynthesis pathway of Penicillium chrysogenum cells, such as penicillin G (PenG), 6-aminopenicillanic acid (6-APA), benzylpenicilloic acid (PIO), ortho-hydroxyphenyl acetic acid (o-OH-PAA), phenylacetic acid (PAA), 6-oxopipeidine-2-carboxylic acid (OPC), 8-hydroxypenicillic acid (8-HPA), L--(-aminoadipyl)-L--cystenyl-D--valine (ACV) and Isopenicillin N (IPN). To correct for matrix effect in ESI and to improve the linearity of calibration lines, the 13C-labeled analogs of the metabolites were added to both sample solutions and the standard calibration mixtures as internal standards. In this approach intracellular samples were analyzed without any sample pretreatment. No recovery control was needed because internal standard mixture was added to the cell samples before extraction process [3]. The 13C-labeled internal standards in this study were extracted from P. chrysogenum cells which were cultured on fully 13C-labeled substrate. The Limit of detections and quantifications were determined for all

these metabolites. This method shows acceptable accuracy and precision in presence of interferences from the sample matrix.

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P151 Session 1, Thursday 1:00 - 2:30 p.m.

Analytical method optimization for a metabolomics study of human amniotic fluid

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Metabolomics analysis of amniotic fluid of genetic amniocentesis might lead to new markers, predictive for preterm birth. This study treats the dedicated optimization of the pre-analytical procedure and the liquid chromatography – mass spectrometry (LC-MS) based analysis. The LC-MS metabolomic profiling was performed using both an optimized reversed-phase (RP, Atlantis dC18 2.1 x 150mm) and hydrophilic interaction (HILIC, TSKgel Amide-80 2.0 x 150mm) type liquid chromatography, coupled with electrospray ionization quadrupole time-of-flight mass spectrometry [1, 2]. As amniotic fluid consists mainly of polar components, HILIC is indispensable in obtaining a wide metabolome coverage.

Initially a comparison of three pre-LC-MS procedures for protein removal (i.e. a molecular weight cutoff (MWCO) polyethersulfone (PES, 3000 MWCO) membrane, a MWCO cellulose triacetate (CTA, 5000 MWCO) membrane, and acetonitrile precipitation) was performed with both LC approaches in the positive ionization mode. PCA score plots show that the membrane filtration methods appear to classify equally well compared to the acetonitrile precipitation method. The acetonitrile precipitation method nevertheless detected much more *m/z*-tR combinations using LC-MS in positive ionization mode than the PES and CTA MWCO membrane methods, on both stationary phases. Moreover, *m/z*-values above 700 Dalton are markedly retained on both membrane filters, which clearly favours the acetonitrile precipitation method for the amniotic fluid analysis.

Subsequently, LC-MS precision, analytical and biological variability were compared on both column types using the acetonitrile based protein removal method. The LC-MS precision on the Atlantis dC18 and HILIC based analyses (8.28% ± 3.56 and 12.57% ± 5.53, respectively; mean R.S.D.% ± S.D. of the peak area of 20 *m/z*-tR features) was comparable with the analytical variability (9.10% ± 3.93 and 10.46% ± 6.52, respectively) demonstrating the reproducibility of the acetonitrile based pre-LC-MS procedure. The biological variability of the amniotic fluid samples analyzed on RP chromatography amounted up to 22.45% ± 18.36, while the biological variability on the HILIC column was 46.22% ± 25.34.

Freeze-drying of the amniotic fluid extracts and subsequent dissolution in a smaller volume of solvent showed, besides an increased number of *m/z*-tR features, much higher variability caused mainly by an increased matrix effect on the HILIC column due to an excess of polar metabolites. In case of the RP analysis, the LC-MS precision increased with 10% when freeze-drying was performed, indicating the chromatographic overloading with these concentrated extracts. The HILIC-based analysis was even incapable in separating the metabolites present in the freeze-dried samples in a reproducible way.

A matrix effect study was performed to investigate the ionization suppression process on both analytical columns by post-column infusion of leucine-enkephaline. The polar metabolite fraction of the amniotic fluid is responsible for the clearly observed matrix effect, resulting in a significant region of ionization suppression at the moment of elution of these components. Especially in the case of HILIC analysis, further improvements are necessary to avoid this matrix effect phenomenon.

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P152 Session 2, Thursday 4:30 - 5:30 p.m.

Enabling targeted metabolomics of blood plasma by using a standardized assay in combination with FIA-MS detection

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Metabolomics has started applying profiling techniques (either by NMR- or MS-based techniques) to investigate the overall composition of biological samples or to identify biological markers. As all "-omics" sciences strive for targeted and quantitative measures during the process of maturation, we sought to create an integrated sample preparation and detection system to foster targeted metabolomics.

A filter-based sample preparation and extraction process was combined with subsequent FIA-MS detection. Not more than 10µl of human blood plasma was processed for each sample point. The whole procedure was automated using liquid handling robotics. An integrated software system was developed to monitor and control the complete assay process, and to calculate concentration values out of the acquired mass spectrometric data.

We conceived and developed an assay using on-filter sample preparation, derivatization, and extraction of biological metabolites. This system, in combination with flow-injection mass spectrometry, is able to generate robust data on more than 150 metabolites from blood plasma samples. The quantified analytes are representatives of these four biologically-interesting classes: acylcarnitines, amino acids, phospho- and sphingolipids and hexoses. A powerful software was used to perform the complex conversion of spectrometric data into concentration values.

The major advantage of the analytical method we have developed is that it enables high-throughput targeted metabolomics in an (optionally) automated fashion. High-throughput targeted metabolomics is vital for the acquisition of reproducible quantitative data. This data is needed to perform routine analyses and to pave the way for metabolomic biomarkers to be used in diagnostics. Although some of the analyzed metabolites did not meet all analytical validation criteria, the overall robustness and reproducibility provided convincing data during field testing. Therefore, this assay can be applied to routine analyses in biomarker-related and pharmaceutical research. Also, studies using other matrices beside plasma have shown promise and we feel this metabolomics method has great potential when applied to other biological systems.

P153 Session 3, Friday 4:00 - 5:30 p.m.

New advances in HRMAS for metabolic profiling of tissues

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Metabolic profiling of biofluids using NMR has received considerable attention due to its potential applications in identifying of biomarkers for diseases. Increasingly complex studies, typically involving large number of biofluid samples, are being conducted due to advances in automation and NMR technology. However, few systematic studies have explored metabolic profiling of tissues despite its promising applications in studying drug absorption and metabolism in tissues. HRMAS NMR has been found to be an excellent technique for conducting tissue profiling due to its reasonable sample requirements, limited sample processing, and quality of the data produced. In our study, we have developed a disposable HRMAS insert to increase the sample throughput and prevent biological contamination of the rotor. This allows the efficient collection, handling, storage, and acquisition of NMR data on tissue samples from various sources. We have examined critical factors influencing a metabolic profiling study such as sample integrity, spectral processing, reproducibility, and quantitation using bovine and murine tissues. We have also compared the nature of information that can be obtained by studying tissues using hrMAS and tissue extracts.

P154 Session 1, Thursday 1:00 - 2:30 p.m.

Global metabolite profiling of carbon metabolism in Mycobacterium tuberculosis: An LC-MS-TOF-based approach

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Tuberculosis (TB) is the leading bacterial cause of deaths worldwide and a public health emergency. A major barrier to control of this pandemic is the lack of understanding of how its etiologic agent, Mycobacterium tuberculosis (MTB), persists in the face of host immunity and chemotherapy. Such knowledge represents a conceptual roadmap to new potential drug targets. Current evidence indicates that MTB adapts metabolism to accommodate the acidic, hypoxic, nitro-oxidative and nutrient-poor conditions of the host niche. Biochemical knowledge of these adaptations at the level of metabolites however remains conspicuously absent.

To better understand the biology of MTB, labeled metabolites of MTB were created by exposing MTB in the logarithmic phase of growth to media containing either U-13C-labeled dextrose or U-2H-labeled acetate. MTB cell extracts were analyzed using accurate mass HPLC/TOF-MS analysis.

Tracking of labeled metabolites was achieved by filtering tentative metabolite identifications on the basis of discordant accurate mass and isotopomer distribution pairs when compared to analytes observed at the same chromatographic retention time in unlabeled biological replicate controls.

P155 Session 2, Thursday 4:30 - 5:30 p.m.

A strategy for increased metabolome coverage in erythrocytes using a single LC chromatographic method with multi-modal LC/MS detection

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Reproducible and comprehensive sample extraction, separation and detection of metabolites with a broad range of physico-chemical properties can be a highly challenging process for LC/MS. Moreover, compound matching to accurate mass libraries based on mass alone is insufficient for identification. We used human erythrocytes to demonstrate a more comprehensive sample extraction method under solvent conditions where the pH has been adjusted to pH 2, pH 6 or pH 9. Furthermore, a single binary solvent chromatographic separation method for LC/MS was developed for ESI and APCI in both ionization modes that incorporated water, methanol and acetate as a mobile phase modifier on a Zorbax SB-aq column. A total of 2,370 features (compounds and associated compound related components: isotopes, adducts and dimers) were detected across all pHs. Broader coverage of the detected metabolome was achieved by observing that (1) performing extractions at pH 2 and at pH 9, leads to a combined 92% increase in detected features over pH 7 alone; and (2) including APCI in the analysis results in a 34 % increase in detected features, across all pHs, than the total number detected by ESI only. A significant dependency of extraction solvent pH on the recovery of heme and other compounds was observed in erythrocytes and underscores the need for a comprehensive sample extraction strategy and LC/MS analysis in metabolomics profiling experiments. In addition we used the single chromatographic system to demonstrate an approach to developing an accurate mass retention time library (AMRT) that significantly improves the metabolite detection coverage and the confidence with which database matches are made. The method has the advantage of the same retention times for metabolites detected by the different ionization methods. A limited sized AMRT erythrocyte library of standards, corresponding to several common metabolites found in erythrocytes, was constructed and used to evaluate actual compound matches from the sample extracts.

P156 Session 3, Friday 4:00 - 5:30 p.m.

QUENCHING OF HUMAN CULTURED SKIN FIBROBLASTS

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Introduction: Metabolomic analysis of cultured cells requires effective removal of all potentially interfering substances from cultivation media with simultaneous metabolic quenching. The procedure needs to be rapid to avoid unnecessary metabolite losses due to cell lysis. We report here an optimized preparation method for metabolomic analysis of human cultured skin fibroblasts.

Methods: We used quenching solutions based on 60% methanol with additions of salts (isotonic concentration; salts removable by freeze-drying e.g. ammonium formiate). Several manipulation techniques of quenching were tested with different volumes of quenching solvent. Quenching procedure was performed either with a pipette or a syringe with a needle. Manipulation techniques were optimized using saturated solution of amidoblack as a dilution marker. Membrane integrity was assessed by the measurement of ATP in the quenching solution and cell extract by capillary electrophoresis.

Results: Salts added to quenching solution does not improve membrane integrity and produce irreproducible debris. Finally quenching and cell extraction was performed with 60% methanol at –40° C. Of all manipulation techniques tested, the best results were achieved by quenching the cells with 15 ml of solution with a syringe with a bent needle. Cultivation flask was held bottom-up and the solvent was sprayed-in and shaked-out continuously during quenching. Another 2 ml of solution was added and cell layer was scraped for extraction. Calculated final volume of culture medium left in the cultivation flask was 2.7 nl, which contributes to intracellular volume by approximately 0.3%. This procedure takes just about 13 seconds and only 11.6 ± 5.8 % (mean ± SD) of cells are lysed.

Conclusion: We have developed a quenching method for cultured human skin fibroblasts. This method is rapid, causes minimal cell leakage and removes cultivation medium very efficiently. We consider this manipulation technique to be optimal for quenching of fibroblasts and possibly other adherent cell cultures. Supported by the grant MSM 6198959205.

P157 Session 1, Thursday 1:00 - 2:30 p.m.

Profiling of amine-containing metabolites by SCX/HILIC

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In metabolomics, liquid chromatography mass spectrometry (LC-MS) is an important technique to profile metabolites in a biological sample. Hydrophilic interaction chromatography (HILIC) is recently becoming popular for the separation of polar metabolites such as sugars or amino acids without the need of derivatization or the use of ion-pair additives to the mobile phase. When using HILIC separation of especially biological samples, care must be taken during sample preparation. For example, a high amount of acetonitrile is required during the loading of the sample, but a high amount of acetonitrile sometimes causes precipitation of polar metabolites. In addition, inorganic salts such as NaCl, which are co-eluted with polar metabolites in HILIC separations, result in interferences with mass spectrometric detection using electrospray ionization. However, removal of salts from a mixture of polar metabolites is still challenging and laborious. Therefore we developed an approach for profiling of polar amine-containing metabolites such as amino acids; in this approach the direct injection of biological sample was possible without removing salts or other contaminants prior to analysis.

In our approach, we used the combination of a SCX precolumn with an amino-based HILIC column. The SCX precolumn was used for trapping polar cationic compounds in ion exchange mode (i.e. a water-rich environment), and then the trapped compounds were desorbed and separated by the SCX-amino-based HILIC column in HILIC mode (i.e. an organic solvent-rich environment), in which the SCX cartridge was used in two different mode. These columns were coupled to an ion-trap MS or an LTQ-Orbitrap MS.

Amino acids were selected as test metabolites. These metabolites were trapped on the SCX precolumn using acidic aqueous condition. After switching the solvent in the cartridge to acetonitrile, all amino acids were successfully eluted and separated by decreasing acetontrile using a basic mobile phase. Using this procedure, inorganic cations were not transferred to the analytical column. Also, the removal of proteins prior to the SCX-HILIC analysis appeared not to be necessary. Endogenous metabolites were successfully analyzed in urine and plasma samples.

Keywords: HILIC, SCX, amino acids

P158 Session 2, Thursday 4:30 - 5:30 p.m.

Use of EDTA to minimize ionic strength dependent frequency shifts in the 1H NMR spectra of urine

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The 1H NMR spectrum of urine exhibits a large number of detectable metabolites and is, therefore, highly suitable for the study of perturbations caused by disease, toxicity, nutrition or environmental factors in humans and animals. However, variations in the chemical shifts and intensities due to altered pH and ionic strength present a challenge in NMR-based studies. With a view towards understanding and minimizing the effects of these variations, we have extensively studied the effects of ionic strength and pH on the chemical shifts of common urine metabolites and their possible reduction using EDTA (ethylenediaminetetraacetic acid). 1H NMR chemical shifts for alanine, citrate, creatinine, dimethylamine, glycine, histidine, hippurate, formate and the internal reference, TSP (trimethylsilylpropionic acid-d4, sodium salt) obtained under different conditions were used to assess each effect individually. EDTA minimizes the frequency shifts of the metabolites that have a propensity for metal binding. Chelation of such metal ions is evident from the appearance of signals from EDTA complexed to divalent metal ions such as calcium and magnesium. Not surprisingly, increasing the buffer concentration or buffer volume also minimizes pH dependent frequency shifts. The combination of EDTA and an appropriate buffer effectively minimizes both pH dependent frequency shifts and ionic strength dependent intensity variations in urine NMR spectra.

P159 Session 3, Friday 4:00 - 5:30 p.m.

Metabolomics approach for biomarker discovery and diagnostic development

Walter Gall (1), Yun Fu Hu (1), Costel Chirila (1), Klaus-Peter Adam (1), Don Rose (1)

(1) Metabolon

Historically, traditional diagnostic development has been a relatively inefficient, long-term process involving discovery of one biomarker at a time, resulting in product development timelines of several years. Genomics and proteomics approaches have experienced challenges with biomarker discovery for diagnostic development, partly due to the vast heterogeneity of populations studied and the requirement for detecting a strong, specific signal amidst wide biological variation. In contrast, a metabolomics approach for identifying biomarker candidates includes measuring a more tractable number of analytes, as well as directly identifying metabolites which more closely reflect the molecular pathways involved in a physiological phenotype or disease mechanism under study.

This paper will present the use of metabolomics in developing a diagnostic test. The process starts with the selection of appropriate clinical samples for identifying relevant biomarkers. These samples are analyzed using a metabolomics platform based on three independent analyses: LC-MS/MS (ESI +), LC-MS/MS (ESI -), and GC-MS. From the raw mass spectral data, biochemicals are identified and quantified using software to filter noise from relevant chemical data and match the spectra to a database generated from reference compounds. After data processing, statistical modeling approaches are implemented on the hundreds of small molecules identified in a single sample, to yield a select group of biomarker candidates that highly correlate to a gold standard reference method for the purpose of developing a diagnostic test. Different subsets of biomarkers are selected

in a variety of combinations when developing a multivariate diagnostic algorithm that may reflect presence of a subclinical condition / disease or its progression. Further analyses of these subsets of small molecules are carried out using targeted, structural elucidation techniques, in preparation for subsequent analytical validation and clinical validation studies for diagnostic product development.

The case study presented here describes one of our first diagnostics in our product development pipeline, QuantoseTM IR (Insulin Resistance) – a simple, fasting blood test that provides a measure of a patient's insulin sensitivity. Insulin resistance has been linked to clinical conditions such as dyslipidemia and hypertension, and is known to precede development of diabetes and cardiovascular disease. Insulin resistance is highly prevalent and estimated to affect a quarter of the U.S. and UK adult population. QuantoseTM IR is being developed as a risk stratification diagnostic test that correlates with the 'gold standard' hyperinsulinemic euglycemic clamp, a cost-, time-, and labor-intensive procedure used by clinical researchers and pharmaceutical companies to measure insulin sensitivity in a subject's peripheral tissues in diabetes and cardiovascular disease research and therapeutic development. In both the clinical research and healthcare market settings, QuantoseTM IR will facilitate detection of asymptomatic, insulin resistant individuals, as well as serve as a complement to existing glycemic control measurements for prediabetics and diabetics. Product profile details including product specification and strategic market positioning of QuantoseTM IR will be discussed.

P160 Session 1, Thursday 1:00 - 2:30 p.m.

Characterisation of the human serum metabolome employing analytical and informatics strategies

Warwick Dunn (1), Paul Begley (1), David Broadhurst (1), Marie Brown (1), Paul Dobson (1)

(1) University of Manchester

Successful metabolic profiling experiments require the chemical identification of 'biologically interesting' metabolites to provide the conversion of raw data to biological knowledge [1]. However, a major dilemma in these studies is that many metabolites are currently unidentified, especially in the more complex mammalian metabolomes containing many thousands of compounds. A range of strategies can be applied to chemically characterise metabolites. These include *ad hoc* identification of biologically interesting metabolites on a per study basis by comparison against a mass spectral or NMR metabolite reference library, or by manual interpretation of mass spectral and/or NMR data. As an alternative the intensive chemical characterisation of complete metabolomes can be performed which provides analytical data to incorporate into libraries and databases that can provide rapid and automated identification of metabolites in future studies. This strategy also provides data pertaining to both the parts list of metabolomes and also indications on the connectivity of these parts in complex metabolic networks. This connectivity and relative changes in metabolic networks with a systemswide approach, compared to single metabolites, is of particular interest when defining differences between healthy and diseased humans [2]. At Manchester we are applying both of these strategies and the latter will be described in detail with regards to its application in the HUSERMET project (www.husermet.org) for the characterisation of the human serum metabolome. In this presentation I will discuss each of the following strategies:

(a) Informatics strategies to define endogenous and exogenous metabolites, previously identified in human serum or those metabolites theoretically present.

(b) Construction of UPLC-MS and GC-MS metabolite libraries from commercially available standards to allow automated and highly specific identification of metabolites. Identification is provided by two

orthogonal properties e.g retention time and EI mass spectrum (GC-MS) and retention time, accurate mass and MS/MS massspectrum (UPLC-MS).

(c) Application of sub-ppm mass accuracies and spectral stitching [3] in UPLC-MS with an LTQ-Orbitrap mass spectrometer, allowing the accurate chemical identification of detected metabolites. such as those not present in mass spectral libraries. Integration with MS/MS and MS3 data provides greater confidence in identifications.

[1] W.B. Dunn, Physical Biology. 2008, 5, 011001.
[2] J. van der Greef, P. Stroobant, R. van der Heijden, Current Opinion in Chemical Biology. 2004, 8, 559-565
[3] A.D. Southam, T.G. Payne, H.J. Cooper, T.N. Arvanitis, M.R. Viant, Analytical Chemistry. 2007, 79, 4595-4602

P161 Session 2, Thursday 4:30 - 5:30 p.m.

Lipids associated with impaired skin barrier function caused by defective ELOVL4 enzyme. Statistical analysis of multidimensional data

Johnie Brown, Susan Leonard, Jeffrey Miller

(1) Applied Biosystems

A metabolomics approach to identify the specific lipid types that are associated with the loss of skin barrier function is presented. Mice born without functioning ELOVL4 have a lethal skin condition that causes them to dehydrate and die within a few hours. The genetic defect prevents the development of normal skin barrier function by preventing the formation of lipids that are important to the structure of the epidermis.

Total lipid extracts were prepared from the skins of mouse littermates representing wild type mice and individuals that were either heterozygous or homozygous for the induced genetic defect in the enzyme ELOVL4. The reverse phase chromatography, the ionization methods, the hybrid Qq-LIT data acquisition methods, the peak detection methods, the statistical analysis methods and the results of this example study are discussed. This work flow represents a general way to acquire and process as much parallel information as possible using the least amount of instrument time. The resulting LC-MS and MS/MS data set can then be used to develop and investigate many hypotheses without additional injections.

P162 Session 3, Friday 4:00 - 5:30 p.m.

Evaluation of the trans-sulfuration pathway by metabolomics and transcriptomics

Laura K. Schnackenberg (1), Minjun Chen (1), Jinchun Sun (1), Ricky Holland (1), Yvonne Dragan

(1) NCTR/USFDA

Reactive drug metabolites are generally detoxified via glutathione conjugation. In this study, urine samples were obtained from studies of six compounds known to cause liver toxicity and glutathione depletion in Sprague-Dawley rats. Based upon the hypothesis that molecules involved in glutathione synthesis would be diminished in order to repopulate glutathione stores, changes in S-adenosylmethionine (SAMe) were evaluated in the urine samples by UPLC/MS. SAMe is the primary source of the sulfur atom in the synthesis of glutathione and was significantly decreased in urine

samples after dosing. In addition to SAMe, the changes in N-methylnicotinate, which is a byproduct of the conversion of SAMe to S-adenosylhomocysteine (SAH) in the glutathione pathway, was investigated by NMR spectroscopy. Similar to SAMe, N-methylnicotinate levels were significantly decreased following dosing. The decrease in urinary N-methylnicotinate was validated by analysis of urine samples collected from studies of three additional liver toxic compounds. Receiver operating characteristic (ROC) curves showed that N-methylnicotinate correlated with histopathology better than alanine aminotransferase or aspartate aminotransferase. Additionally, microarray data from rat liver samples that had been treated with liver toxins that generated reactive metabolites and caused glutathione depletion were obtained from the Gene Expression Omnibus (GEO) database. Some genes involved in the trans-sulfuration pathway were found to be significantly decreased following dosing. The results show that N-methylnicotinate and SAMe are potential non-invasive preclinical biomarkers of toxicity related to glutathione depletion for detoxification of reactive drug metabolites.

P163 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolite concentrations are coordinated with the expression of functionally related genes in Saccharomyces cerevisiae.

Patrick Bradley (1), Matthew Brauer (1), Jie Yuan (1), Olga Troyanskaya (1), Josh Rabinowitz (1)

(1) Princeton University

While cases of mutual regulation by metabolites and gene products have been and continue to be described, identifying the full scope of these interactions is important for improving rational control of metabolism to meet therapeutic and bioengineering objectives. An experimental method with the potential to reveal these interactions is the simultaneous measurement of metabolite and transcript concentrations. However, as metabolites that are only distantly related in terms of biochemical transformations can nevertheless show highly correlated concentration changes, straightforward interpretations of this type of data can be difficult to realize. Moreover, whether functionally-related metabolites and transcripts in fact show coherent patterns of concentration changes remains an unresolved question.

In the present work, we have subjected S. cerevisiae to carbon and nitrogen deprivation and have measured the dynamic responses of metabolite and transcript concentrations. We demonstrate that the metabolomic and transcriptional responses are indeed coordinated, and show that this coordination depends on metabolite class (for instance, glycolytic compound or amino acid) and experimental context (that is, the perturbation to which the cells were subjected), parameters whose significance has not been described. Furthermore, we show that by accounting for these parameters using a novel Bayesian approach, specific metabolite-gene interactions can be identified from the data. This algorithm effectively predicts known enzymatic and regulatory relationships, including a gene-metabolite interaction central to the glycolytic-gluconeogenic switch.

P164 Session 1, Thursday 1:00 - 2:30 p.m.

Mapping metabolic alterations in rat lungs and blood plasma after exposure to environmental tobacco smoke

Dinesh Kumar (1), Tobias Kind (1), Carol Hood (2), Kent Pinkerton (2), Oliver Fiehn (1)

(1) UC Davis Genome Center (2) UC Davis Center for Health and the Environment

Metabolomics results are geared to convey a biological view of differential regulation. However, the finding of statistical differences and multivariate clusters of altered metabolites do not easily reveal functional relationships between such compounds. Therefore, interpretation of differential regulation of the identified metabolites should be guided by biochemical mapping to be of biomedical relevance. These maps should include preferentially all metabolic signals, but at least all compounds that were reported with structural identifications. In samples from perfused lungs and blood of rats that were exposed to sidestream tobacco-smoke, Up to 140 compounds were identified using the Fiehnlab GCTOF retention index based MS libraries. We compare efforts to map these metabolite structures to functionally meaningful graphs. Timed pregnant rats were subjected to environmental tobacco smoke daily at 1 mg/m^3 for 6 hours each day in controlled chambers from gestation day 5 to gestation day 20 (term is 21 days). PBS-perfused rat lungs were fresh frozen prior to homogenization and extraction. GC-TOF mass spectrometry (Leco Pegasus IV) was performed using Gerstel automatic liner exchange and cold injection conditions. Blood plasma samples were prepared by cold precipitation/extraction using degassed –20°C isopropanol / acetonitrile / water (3:3:2). Quantitative results were statistically evaluated, and significant differences were mapped to biochemical and chemical databases by open-access tools. A variety of open access tools from KEGG, Reactome, MetaCyc and ARM were used to map differential regulation of metabolites to biochemical network graphs. Pathway and reaction networks were downloaded as SBML and Biopax formats or created in Cytoscape SIF network format. Additionally, chemical structures were employed to calculate chemical similarity indices which were visualized by their resulting distances. For visualization the size of node in cytoscape was adjusted according to the results of t-test analysis (p-value or x-fold change). Three different approaches were compared for visualizing experimental results: mapping to standard biochemical databases, visualization by chemical structure similarities and a hybrid approach employing atomic reconstruction of metabolism (ARM). The visualization results were then evaluated by the number of metabolic nodes that were represented in network graphs and the topology of network that should aid the biological interpretation. Only 50% of the identified metabolites were represented by Reactome DB queries, 60% by MetaCyc queries and over 75% by KEGG and ARM queries. These results indicate that many identified metabolites lack annotated biochemical pathways in animals. In an alternative approach, network graphs were created based on Tanimoto chemical distances using molecule substructure matrices. Constrained by similarity thresholds, functionally related clusters of metabolites were found that resembled the clustering of lipids, carbohydrates, TCA cycle intermediates, aromatics, amino acids and urea cycle intermediates mapped by the ARM database. Clear differences in metabolic alterations were discovered between acute and chronic smoke exposures, pointing to a recovery phase at which few alterations were marked. This work was funded by NIEHS R01 ES 13932.

P165 Session 3, Friday 4:00 - 5:30 p.m.

Characterization of dissolved organic matter in marine pore waters by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry

Frauke Schmidt (1), Boris Koch (2), Marcus Elvert (1), Matthias Witt (3), Gabriela Zurek (3)

(1) Bremen University (2) Alfred-Wegener-Institute Bremerhaven (3) Bruker Daltonik

Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICR-MS) has been recently been used for the characterization of dissolved organic matter (DOM). This technique is applied to DOM of marine pore waters in order to obtain insights into the fate of organic matter (OM) shortly after its deposition onto the shelf. Continental margins are the major reservoir of organic matter preservation and recycling, but these processes are complex in nature and difficult to assess. Variations in OM sources and biogeochemical processes in terrestrial as well as marine environments require an extended scope of investigation. We combine DOM analysis by FT-ICR-MS with the well established analysis of lipid biomarkers in particulate organic matter (POM) of the associated sediments.

All samples derive from shelf sediments and local river waters of Galicia (north-western Iberian margin). Sediment pore waters were obtained by rhizon sampling. DOM was extracted from the sediment pore waters and filtered river waters by solid phase extraction. DOM samples were analyzed on a 9.4 T FT-ICR mass spectrometer coupled to an electrospray ionization source in negative ion mode. Unequivocal molecular formulas were calculated on the base of the exact masses. Lipid biomarkers were obtained from the sediment phase by solvent extraction. After separation by gas chromatography, the main compounds were identified and quantified by mass spectrometry and flame ionization detection (FID), respectively.

FT-ICR-MS analyses resulted in numerous molecular formulas, representing the wide range of different molecules in pore and river water DOM. Weighted average molecular element ratios of O/C and H/C were calculated from peak intensity in the FT-ICR-MS data and visualized in van Krevelen plots. Marine DOM in sedimentary pore waters showed high H/C and low O/C ratios compared to riverine DOM. These values either represent the different source signals of the original POM or indicate a higher DOM degradation in the pore waters, which would be consistent with the release of H-rich DOM from fresh marine OM by microbial degradation. Double-bond equivalents (DBE), i.e. the sum of rings and double bonds in a molecule, and the size of the average DOM molecule in every individual sample gave evidence for a high microbial activity in sediments, specifically observed for sediments with a high accumulation of fresh material (e.g. in the mid-shelf mudbelt).

Presence/absence cluster analyses of the individual samples revealed that sampling sites with relatively similar features in their lipid biomarker distribution differ with respect to their molecular DOM composition. Lipid compounds in the corresponding POM provided information about the different sources as well as the degradation state of OM. We hypothesized that labile DOM fractions are more rapidly turned over by sedimentary microorganisms, whereas POM is more refractory. Hence, the POM source signal is still preserved in the sediments, with degradation becoming more important during longer residence time along the main transport pathways.

P166 Session 1, Thursday 1:00 - 2:30 p.m.

Multinuclear NMR Metabolomic Analysis of 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) Elicited Hepatotoxicity in Rats and Mice

Gary Jahns (1), Michael Kent (2) , Meghan Makley(2), Ania Kopec (3) , Lyle Burgoon (3)

(1) BAE Systems USA (2) Wright State University USA (3) Michigan State University

Our research team has used 1H, 13C, and 31P nuclear magnetic resonance (NMR) spectrometry to comprehensively analyze aqueous and lipid liver extracts obtained from control and TCDD-treated animal models, for the purpose of investigating prior observations of species-specific hepatotoxic responses. 31P analysis of aqueous extracts identified various phosphomono- and phosphodi-esters, while 31P analysis of lipid extracts provided a measure of the six major liver phospholipids. 13C NMR of lipid samples produced more complex spectra with ~280 detectable peaks, of which 107 have been identified within 6 metabolite categories. We have previously reported a 13C NMR data processing and analysis methodology that 1) locally aligns spectra by cross-correlation of samples; 2) identifies data clusters via Principal Components Analysis; 3) produces mean spectral estimates from cluster centroids; 4) measures mean-spectral peak amplitude ratios; and 5) determines confidence levels of amplitude ratios from order statistics. Spectral resolution is maintained by this methodology while effects due to anomalous chemical shifts resulting from sample environmental factors are minimized. Proton (1H) spectra of aqueous liver samples has proved to be even more challenging with many overlying peaks, requiring new multivariate data processing methodologies that are currently under evaluation. To investigate species-specific hepatotoxicity, these NMR spectra were used to assess the metabolomic responses to TCDD, referenced to vehicle-treated controls, in immature ovariectomized C57BL/6 mice (30 ug/kg) and Sprague-Dawley rats (10 ug/kg). We found that cardiolipin decreased with exposure in both species, suggesting that both species may experience TCDD-mediated mitochondrial toxicity. But otherwise mice exhibit a more pronounced liver lipid effect than rats with significant increases in five of the six lipid categories observed with 13C NMR. Rats, however, showed an increase in phosphocholine (per 31P aqueous NMR) and decrease in sphingomyelin (per 31P lipid NMR), which was not seen in mice. The implications of these results for the metabolic processes elicited by

P167 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolomics approach to study bile acids metabolism as index of toxicological effects in humans

Giuseppe Giordano (1), Fabiano Reniero (1), Lino Chiandetti (1), Claude Guillou (1)

(1) European Commission, JRC, IHCP – Ispra

The primary bile acids (the taurine and glycine conjugates of chenodeoxycholic acid and cholic acid) are synthesised from cholesterol in the liver. The first and rate limiting step in the synthetic pathway, cholesterol 7-hydroxylase, is inhibited by bile acids as they flow through the liver in the enterohepatic circulation. Bile acids also reduce hepatic sterol synthesis by feedback inhibition of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) reductase. Secretion of bile acids from the hepatocytes into the canaliculi drives water secretion so that a major component of bile flow is “bile acid dependent”. In the intestinal lumen, bile acids are responsible for assisting the digestion and absorption of lipids. Liver is an organ acting as a filter for the body to exposition to various xenobiotics. Presence of bile acids in urine is indicative of a “no-healthy” status for the patient. We

present the results of a study on intrahepatic cholestasis of pregnancy (ICP), which women can develop during their third trimester of pregnancy. Complications in ICP are increased risks of preterm labor, preterm birth, and abnormal fetal heart rate (FHR) tracings and spontaneous preterm labor and fetal death, in 3% to 4% of cases. We applied metabonomic approach based on an analytical protocol for LC/MS-MS metabolic profiling of urine samples. Multivariate statistical approach was applied to extract information from complex spectra. This approach permitted to evidence in particular one biomarker highly correlated with the pathology. The structural identification is in progress: this molecule could be used in a target analysis as markers for the early detection of the pathology.

P168 Session 3, Friday 4:00 - 5:30 p.m.

Endogenous metabolite markers of jet fuel (Jet A) inhalation exposure

Nestor Tarragona (1), Harry Luithardt (2), Jose Halperin (3), Mark Westrick (4), Erin Wilfong (5)

(1) Tufts Medical Center (2) Solutions Labs Inc (3) Harvard Medical School (4) United States Air Force Academy (5) Naval Health Research Center Detachment

Approximately 230 billion liters of military Jet Propulsion Fuel-8 (JP-8, domestic; F-34, international) and the commercial jet industry equivalents Jet A (domestic) and Jet A-1 (international flights) are consumed worldwide on an annual basis (100 billion liters in the US). Military and civilian personnel are occupationally exposed to high levels of jet fuel each year, and published studies have reported both short-term and persistent health effects, including neurological impairments and pulmonary and immune responses following acute and chronic jet fuel exposure. Short-term exposure limits of 1000 mg/m3 and an 8-h permissible exposure limit of 350 mg/m3 exist presently, but there is little flightline, air-hangar, or within-aircraft monitoring of exposure levels, and there is minimal use of personal protection devices. While xenobiotic exposure markers such as 1- and 2-naphthol concentrations in human urine have been proposed, their interpretation is obscured by other possible origins (smoking or other chemicals) and the probability that chemical constituents of jet fuel (over 200 compounds) may have biologically synergistic or antagonistic effects. Metabolomics may contribute alternatives for providing other useful markers with respect to both health monitoring and understanding the biochemistry of exposure. We present a study designed to accurately simulate jet fuel exposure to aircraft personnel by exposing rats during their waking hours to compensate for circadian effects. Female Sprague-Dawley (n=30) rats (7-8 weeks of age) obtained from Charles River Laboratories were assigned to one of five exposure groups (n=6/group) and exposed to 500, 1000, and 2000 mg/m3 of a mixed vapor/aerosol Jet-A atmosphere or air (controls) for 4 hours/day for 14 consecutive days. Another naïve group of animals were separately housed for the study duration. Urine was collected daily, and prepared for metabolic profiling with positive ionization mode UPLC-MS. Statistical analysis adjusted for multiple hypothesis testing was applied to identify small molecule markers to distinguish the control and the high dose groups. Statistically significant up-regulated and down-regulated compounds (some of endogenous origin) were found to discriminate between these groups. We present these findings along with several immune, pulmonary, and histology end points that provide a biological context (measurements made include total protein, lactate dehydrogenase, beta glucuronidase, cytokines, organ weights, serum chemistry, and hematology).

P169 Session 1, Thursday 1:00 - 2:30 p.m.

NMR and MS analysis of hemolymph from pollutant exposed Daphnia magna

Joshua Hicks (1), Helen Poynton (2), Nadine Taylor (3), Seonock Woo (4), Leona Scanlan (5)

(1) Bruker-BioSpin (2) EPA (3) UC, Berkeley (4) UB, Berkeley (5) Birmingham University

As an index organism, toxicity response by Daphnia magna to toxicants in the ecosystem is typically monitored by acute or chronic lethality assays. Identifying metabolite and secondary metabolite profile changes in the presence of toxic stressors provides a method of systematically monitoring the biological response without using such termination endpoints where the exposure levels do not represent environmentally relevant levels of toxicants. Previously it was demonstrated that NMR can be used as a tool to distinguish exposure to different metal toxicants and subsequent principal component analysis (PCA) was able to target several chemical shifts which display intensity fluctuations corresponding to changes in the metabolic profile. Improved NMR techniques provide a faster method of acquiring data and provide new evidence into metabolite identification. In a subsequent cadmium-exposure study, direct infusion Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry was used to profile the polar metabolome of D. magna hemolymph. Following extensive processing of the mass spectra, PCA was conducted on generalized log transformed peak intensities and revealed clustering of the control and Cd-exposed organisms. This result is consistent with the NMR spectral analysis and a combined metabolite profile analysis is demonstrated.

P170 Session 2, Thursday 4:30 - 5:30 p.m.

Metabolomics and biomarker discovery: NMR spectral data of urine and hepatotoxicity by carbon tetrachloride, acetaminophen, and D-galactosamine in rats

Kyu-Bong Kim (1), Myeon Woo Chung (1), So Young Um (1), Ji Sun Oh (1), Seon Hwa Kim (1)

(1) Pharmacology Department, National Institute of Toxicological Research, Korea Food and Drug Administration

The primary objective of this study was to discover biomarkers which are correlated with hepatotoxicity induced by chemicals using 1H NMR spectral data of urine, plasma, and liver. A procedure of nuclear magnetic resonance (NMR) urinalysis using pattern recognition was proposed for early screening of hepatotoxicity of CCl4, acetaminophen (AAP), and D-galactosamine (GalN) in rats. Those compounds were expected to induce necrosis to hepatocytes and this was confirmed through blood biochemistry and histopathology. CCl4 (1 ml/kg, po) and GalD (0.8 g/kg, ip) were single administered to Sprague-Dawley (SD) rats and urine was collected every 24 h pre-dose and 2 days post-dosing. Animals were sacrificed 24 h or 48 h post-dosing and blood and liver were collected for 1H NMR analysis. AAP (2 g/kg, po) was administered for 2 days and then the animals were sacrificed 24 h after last treatment. Urinary, plasma, and hepatic 1H NMR spectroscopies revealed evidently different clustering between control groups and hepatotoxicants treatment groups in metabolic profilings through principal component analysis (PCA) and partial least aquare (PLS)-discrimination analysis (DA). In targeted profilings, endogenous metabolites of allantoin, citrate, taurine, 2-oxoglutarate, acetate, lactate, betaine, succinate, phenylacetate, 1-methylnicotineamide, hippurate, and benzoate were selected as biomarkers for hepatoxicity by CCl4, AAP, and GalN in urine. Comparison of rat 1H NMR PCA and PLS-DA data with histopathological changes suggest that 1H NMR urinalysis can be used to predict hepatotoxicity induced by CCl4, AAP, and GalN.

P171 Session 3, Friday 4:00 - 5:30 p.m.

GC-MS-based Metabolomic Analysis of Hepatic Lipid Composition in TCDD-treated C57BL/6 Mice

Bryan Mets (1), Lyle Burgoon (1), Tim Zacharewski (1)

(1) Michigan State University

2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD), the most potent of the dioxins, is an environmentally persistent contaminant that elicits a variety of toxic and biochemical effects mediated by the aryl hydrocarbon receptor (AhR). This includes inducing a fatty liver in mice that may model aspects of human metabolic syndrome. Using an immature, ovariectomized C57BL/6 mouse model, we have shown that 30 μg/kg TCDD disrupted gene expression associated with lipid metabolism and transport. Treated mice also exhibited increased serum triacylglycerides at 24 and 168 hrs post-exposure, and hepatic steatohepatisis at 72 and 168 hrs. Lipid extracts were derivatized to fatty acid methyl esters to facilitate GC-MS profiling of TCDD-mediated hepatic changes in lipid composition compared to time-matched vehicle treated animals. The levels of saturated fatty acids, such as stearic acid (C18:0), and arachidic acid (C20:0), did not change compared to controls, whereas their monounsaturated metabolites, such as oleic acid (C18:1n-9; 1.5x at 24hrs and 2.0x at 72hrs) and C20:1 (2.0x at 24hrs, 4.0x at 72hrs), increased in a time-dependent manner. Other desaturated metabolites also exhibited various TCDD-mediated changes, suggesting that AhR-mediated regulation of lipid transport and metabolism gene expression has significant consequences on hepatic lipid accumulation and composition.

This work was supported by the National Institute of Environmental Health by grant R01ES013927

P172 Session 1, Thursday 1:00 - 2:30 p.m.

Metabolomic profiling of lipids in wild type versus yeast cadmium factor mutant strains of Saccharomyces cerevisiae

Timothy Waybright, Gary Muschik, Que Van, Michael Dean, Michael Citro, Timothy Veenstra

Laboratory of Proteomics and Analytical Technologies, SAIC-Fredreck, Inc

The YCF1-ADE2 wild type and ycf1-ADE2 mutant (yeast cadmium factor protein which is a MRP transport mutant) strains of Saccharomyces cerevisiae were extracted with methanol-water and analyzed using C4 reverse phase high-performance liquid chromatography (HPLC) coupled with electrospray ionization mass spectrometry (ESI-MS). Extracts were analyzed to detect and identify differences in the lipid metabolite fingerprint between the two strains. Glutathione conjugates and other unknown substrates are transported by YCF1. These transport proteins are encoded by the yeast ABC1 gene and are related to the human ABCC gene subfamily of MRP transporter proteins. Since mutations in the human ABCC6 gene are associated with the development of Pseudoxanthoma elasticum (PXE) in humans, the analysis of yeast metabolites may lead to a better understanding of the molecular processes and metabolic pathways in patients afflicted with PXE.

P173 Session 2, Thursday 4:30 - 5:30 p.m.

Hepatotoxicity of chlorpyrifos in zebrafish liver cells by NMR-based metabolomics

Wenlin Huang (1), Quincy Teng (1), Drew Ekman (1), Timothy Collette (1)

(1) Environmental Protection Agency

For decades chlorpyrifos (CPS) has been one of the most widely used organophosphate insecticides for a variety of agricultural and public health applications. The extensive use of CPS inevitably results in exposure to a small number of the human population. It is believed that the chemical is metabolically oxidized to its oxygen analog, chlorpyrifos oxon (CPO), which can cause acute toxicity in humans by the inhibition of acetylcholinesterase in the central nervous systems. The metabolical oxidization of CPS to CPO primarily occurs in livers by multiple cytochrome P450 (CYP) enzyme isoforms. The aim of this study is to identify the metabolic changes in zebrafish liver cells (ZFL) caused by CPS and CPO at different dose levels and to investigate any differences in the cells response to the exposure of the two chemicals. This study is also a proof-of-concept for in-vitro metabolomics using fish cell culture as a model for testing chemical toxicity.

To study the hepatotoxicity of CPS and CPO, we conducted the exposure of ZFL cells to the chemicals with two dose levels of 0.5 and 50 ppb. The cells were quenched at 24 hours and 72 hours and extracted using a two phase method. ^{1}H NOESY NMR spectra were acquired on the polar extracts and subjected to multi-variable statistical analysis. The preliminary statistical analysis indicated that the oxidization of CPS in ZFL cells did not take place at the low dose level, while CYP enzymes were activated by CPS at the high dose level, which oxidized the CPS molecules. The study demonstrates that NMR-based metabolic profiling using cell cultures can provide a quick and effective tool to test the toxicity of chemicals. In addition, we will report the NMR metabolic profile of ZFL intracellular extracts determined by 2D TOCSY and HSQC experiments. The changes of metabolic profile in the ZFL cells caused by CPS and CPO will also be presented.

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