1st Nordic Metabolomics Conference
August 26–28, 2018 Örebro, Sweden

ÖREBRO UNIVERSITY

PROGRAM

1st Metabolomics Conference 26–28 August 2018
Venue: Aula Nova, Örebro University

SUNDAY, AUGUST 26

15:00	Poster set-up
15:00–17.30	Registration opens
18:00	Conference opening – greetings from Nordic Metabolomics Society, organizing committee and Örebro University (Professor Johan Schnürer, vice-chancellor)
18:15	Opening keynote lecture: Antonio Vidal-Puig (University of Cambridge, UK) *"Adipose tissue expandability, lipotoxicity and the metabolic syndrome"*
19:00	Welcome reception

MONDAY, AUGUST 27

06:45–07:30	Morning jog/morning walk in Örebro (sponsored by Larodan AB). Meeting point Scandic Grand Hotel
09:00–10:30	**Session 1 – Metabolomics workflows and data analysis** Chair: Thomas Moritz (Umeå University, Sweden)
09:00	Keynote lecture: Gary Siuzdak (Scripps Research Institute, USA) *"Activity metabolomics"*
09:30	Roland Nilsson (Karolinska Institute, Sweden) *"A web service framework for interactive analysis of metabolomics data"*
09:50	Pär Jonsson (Umeå University, Sweden) *"Extensions and improvements of the OPLS framework exemplified in metabolomics data"*
10:10	Maria Anastasiadi (Cranfield University, UK) *"Application of machine learning methods to predict usage, age, and harvest season in 66 apple cultivars"*
10:30–11:00	Break and posters
11:00–12:00	Panel debate and Q&A – Metabolomics workflows: quality control, data processing, metabolite identification and quantification
	Panel discussion *Panelists:* Tuulia Hyötyläinen (Örebro University, Sweden) Steffen Neumann (Leibniz Institute of Plant Biochemistry, Germany) Tomas Pluskal (Whitehead Institute, USA) Garry Siuzdak (Scripps Research Institute, USA) *Moderator*: Matej Orešič (Örebro University, Sweden and University of Turku, Finland)
12:00–13:30	Lunch and posters

13:30–15:00	**Session 2 – Genome-scale metabolic modelling to study health and disease** Chair: Rikard Landberg (Chalmers University of Technology, Sweden)
13:30	Keynote lecture: Adil Mardinoglu (SciLifeLab, Sweden) "The employment of systems biology in the treatment of liver diseases"
14:00	Saeed Shoaie (King's College London, UK) "Systems biology of oral and gut microbiome in health and diseases"
14:20	Óttar Rolfsson (University of Iceland, Iceland) "Metabolic systems analysis of epithelial to mesenchymal cell transition in breast epithelium"
14:40	Partho Sen (University of Turku, Finland) "Genome-wide metabolic modelling of human CD4+ T-helper cell"
15:00–15:30	Break and posters
15:30–17:00	**Session 3 – Molecular physiology in health and disease** Chair: Stine Marie Ulven (University of Oslo, Norway)
15:30	Keynote lecture: Matej Orešič (Örebro University, Sweden and University of Turku, Finland) "Metabolome en route to autoimmunity and overt disease: prospective studies in type 1 diabetes and celiac disease"
16:00	Tianrong Yeo (University of Oxford, UK) "Utility of NMR plasma metabolomics as a diagnostic biomarker in antibody-mediated inflammatory demyelinating diseases of the central nervous system"
16:20	Maaria Kortesniemi (University of Turku, Finland) "Human milk metabolomes are shaped by maternal psychological distress and milk cortisol levels"
16:40	Masoumeh Alinaghi (Aarhus University, Denmark) "Bovine colostrum modifies the metabolic response during neonatal bloodstream sepsis in preterm pigs"
18:30	Guided tur at the castle
19:15	**Conference dinner** General Assembly of the Nordic Metabolomics Society

09:00–10:30	**Session 4 – Food and health** Chair: Hanne Christine Bertram (Aarhus University, Denmark)
09:00	Keynote lecture: Lorraine Brennan (University College Dublin, Ireland) *"Metabolomics in nutrition – current status and future outlook"*
09:30	Lin Shi (Chalmers University of Technology, Sweden) *"Biomarkers of fish intake and their association with type 2 diabetes risk"*
09:50	Stefania Noerman (University of Eastern Finland, Kuopio, Finland) *"Metabolic profiling of high egg consumption and the associated lower risk of type 2 diabetes in middle-aged Finnish men"*
10:10	Patrik Hansson (University of Oslo, Norway) *"Gender differences in postprandial response to different dairy products on lipoprotein subclasses: a randomized controlled cross-over trial"*
10:20	Rebekka Thøgersen (Aarhus University, Denmark) *"Metabolomics as a tool to elucidate the effect of inulin fortification of processed meat – a rat intervention study"*
10:30–11:00	Break and posters
11:00–12:20	**Session 5 – Industry session** Chair: Lars Dragsted (University of Copenhagen, Denmark) Steven Fischer (Agilent Technologies) *"Investigation of pyrazinamide mechanism of action for tuberculosis using metabolomics"* Joanne Connolly (OMICS Business Development, Waters Northern Europe) *"A non-targeted metabolomic study of retail pomegranate juice products to investigate nutritional and quality characteristics – using a novel data independent acquisition mode and ion-mobility on a QTof MS instrument"* Anas Kamleh (Thermo Fisher Scientific) *"Improved metabolome coverage and increased confidence in unknown identification through novel automated acquisition strategy combining sequential injections and MSn"* Aiko Barsch and Manfred Spraul (Bruker) *"Novel and innovative Solutions for advancing Metabolomics and Lipidomics research"*
12:20–13:20	Lunch and posters

13:20–14:50	**Session 6 – Environmental and plant metabolomics** Chair: Kati Hanhineva (University of Eastern Finland, Finland)
13:20	Keynote lecture: Stephen M. Rappaport (University of California Berkeley, USA) *"Metabolomics of Neonatal Blood Spots Reveals Lipids Associated with Childhood Leukemia"*
13:50	Tuulia Hyötyläinen (Örebro University, Sweden) *"Exposure to environmental chemicals during pregnancy affects the lipidomic profiles in the offspring and may mediate the risk of type 1 diabetes"*
14:10	Ilara G.F. Budzinski (Swedish University of Agricultural Sciences) *"Metabolite characterization along wood forming tissues in hybrid aspen"*
14:30	Tomáš Pluskal (Whitehead Institute for Biomedical Research, USA) *"The biosynthetic origin of psychoactive kavalactones in kava"*
14:50	Closing and awards

GOOD TO KNOW DURING THE CONFERENCE

Questions during the conference
❱ Registration desk in the Nova Building Lounge
❱ E-mail to konferensinfo@oru.se
❱ Telephone +46 702571312

Conference buses
From Nova Building to Elite Stora Hotellet and Scandic Grand Hotel
Sunday August 26 at 20.30 and 21.00
Monday August 27 at 17.15
Tuesday August 28 at 15.15 – to Elite Stora Hotellet and Resecentrum

From Scandic Grand Hotel and Elite Stora Hotellet
Monday August 27 at 08.30
Tuesday August 28 at 08.20 (luggage day)

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Matej Orešič (chair and local host; Örebro University, Örebro, Sweden
and University of Turku, Turku, Finland)

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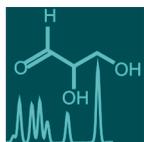
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Editor-in-Chief

Prof. Dr. Peter Meikle
Metabolomics Laboratory NHMRC, Baker Heart and Diabetes Institute, Melbourne, Victoria 3004, Australia

Message from the Editor-in-Chief

The metabolome is the result of the combined effects of genetic and environmental influences on metabolic processes. Metabolomic studies can provide a global view of metabolism and thereby improve our understanding of the underlying biology. Advances in metabolomic technologies have shown utility for elucidating mechanisms which underlie fundamental biological processes including disease pathology. *Metabolites* is proud to be part of the development of metabolomics and we look forward to working with many of you to publish high quality metabolomic studies.

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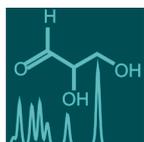
Metabolomics — Workflows, Methods and Applications

Guest Editors:

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Deadline for manuscript submissions:
1 February 2019

Message from the Guest Editors

Dear Colleagues,

The 1st Nordic Metabolomics Conference is the inaugural annual meeting of the newly established Nordic Metabolomics Society. The conference aims to highlight and discuss the latest metabolomics research in the Nordic countries and abroad.

We welcome papers focused on metabolomics workflows, metabolite identification and quantification, metabolomics in nutrition, environmental and plant metabolomics, and particularly, but not exclusively, extended conference papers from the Nordic Metabolomics Conference 2018.

More information about the Nordic Metabolomics Conference 2018 can be found at: https://www.oru.se/NordicMetabolomicsConference

Prof. Dr. Matej Orešič
Prof. Dr. Hanne Christine Bertram
Prof. Dr. Lars Ove Dragsted
Prof. Dr. Tuulia Hyötyläinen
Prof. Dr. Rikard Landberg
Prof. Dr. Stine Marie Ulven
Guest Editors

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Special Issue

ABSTRACT BOOK

ORAL TALK

K1

Adipose tissue expandability, lipotoxicity and the metabolic syndrome

Antonio Vidal-Puig

University of Cambridge, Metabolic Research Laboratories, Wellcome Trust-MRC Institute of Metabolic Science, Addenbrooke's Hospital, Cambridge, CB2 0QQ

The link between obesity and type 2 diabetes is clear on an epidemiological level, however the mechanism linking these two common disorders is not well defined. One hypothesis linking obesity to type 2 diabetes is the adipose tissue expandability hypothesis. The adipose tissue expandability hypothesis states that a failure in the capacity for adipose tissue expansion, rather than obesity per se is the key factor linking positive energy balance and type 2 diabetes. All individuals possess a maximum capacity for adipose expansion which is determined by both genetic and environmental factors. Once the adipose tissue expansion limit is reached, adipose tissue ceases to store energy efficiently and lipids begin to accumulate in other tissues. Ectopic lipid accumulation in non-adipocyte cells causes lipotoxic insults including insulin resistance, apoptosis and inflammation. This article discusses the links between adipokines, inflammation, adipose tissue expandability and lipotoxicity. Finally, we will discuss how considering the concept of allostasis may enable a better understanding of how diabetes develops and allow the rational design of new anti diabetic treatments.

Activity Metabolomics: Identifying Metabolites that Modulating Phenotype

Gary Siuzdak, The Scripps Research Institute, La Jolla, CA, USA

The metabolome interacts with, and hence coalesces, all the omic layers. Therefore, it is not surprising that we have started to recognize the biological activity of metabolites as an integral part of metabolomic studies. When systems biology evolved as a biological discipline, with the express purpose of comprehensively describing all the biochemical reactions of an organism, metabolomics was rapidly recognized as an important component, allowing us to confirm computational predictions and shed light on biochemical pathways. However, it is only recently that the concept of metabolomics activity screening (MAS)[1] has started to integrate metabolomics, systems biology, and bioactivity. The field is in its infancy, yet the practical impact of identifying novel bioactive metabolites and characterizing their associated control, function, and mechanisms of action is exciting in their ability to modulate phenotype.

As with any new scientific endeavor, MAS needs to overcome several significant challenges, including the need to develop effective approaches to identify active metabolites. As outlined in this presentation, there are multiple approaches to accomplishing this, however the process is challenging because metabolic activity will likely vary depending on the organism and the phenotype of that organism. This concept is particularly intriguing as it transforms our view of the metabolome, which instead of a phenotypic descriptor it is a phenotype modulator. Or better yet, as metabolomics scientists using metabolites, we are no longer passive observers and instead active participants in modulating the system we are studying.

[1] Metabolomics Activity Screening Identifies Metabolites that Modulate Phenotype
C. Guijas, J.R. Montenegro-Burke, B. Warth, M.E. Spilker, G. Siuzdak
Nature Biotechnology 2018, 36 (4), 316-320.

1st Nordic Metabolomics Conference
Örebro, August 26-28, 2018

ABSTRACT

Title
A Web Service Framework for Interactive Analysis of Metabolomics Data

Authors
Yaroslav Lyutvinskiy[1,2,3], Jeramie Watrous[4], Mohit Jain[4], Roland Nilsson[1,2,3]

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Abstract text

Analyzing mass spectrometry-based metabolomics data presents a major challenge to metabolism researchers, as it requires accessing and processing large data volumes through complex "pipelines", even in cases where only a single metabolite or peak is of interest. This presents a significant hurdle for data sharing, reanalysis or meta-analysis of existing data sets, whether locally stored or available from public repositories. Here we describe a fundamentally different approach to mass spectrometry data analysis based on mzAccess (1), a new software system that provides interactive, online access to primary mass spectrometry data in real time via a web service protocol, circumventing the need for bulk data download, file format conversion and processing. mzAccess allows users to directly query remotely stored instrument data for spectra, chromatograms, or two-dimensional MZ-RT areas in either profile or centroid modes through a simple, uniform interface that is independent of vendor or instrument type. Using a cache mechanism, mzAccess achieves response times in the millisecond range on high speed internet connections for typical LC-MS peaks, enabling real-time browsing of large data sets with hundreds or even thousands of samples. The mzAccess service is readily accessible from most programming languages and data analysis platforms, and can be integrated into existing analysis software as a data access layer. The system is freely available at www.mzaccess.org. By simplifying access to metabolite data, we hope that this system will help enable data sharing and reanalysis in the metabolomics field.

(1) Yaroslav Lyutvinskiy, et al. A Web Service Framework for Interactive Analysis of Metabolomics Data. Analytical Chemistry, 89:5713–5718, 2017.

1st Nordic Metabolomics Conference
Örebro, August 26-28, 2018

Title
Extensions and improvements of the OPLS framework exemplified in metabolomics data.

Authors
Pär Jonsson[1], Henrik Antti[1]

Affiliation
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Abstract
Chemometric methods are commonly used in metabolomics to find relationship between metabolite patterns and phenotypical differences or changes. One of the most frequently used chemometric tools is orthogonal projections to latent structures (OPLS)[1], especially OPLS Discriminant Analysis (OPLS-DA). OPLS can be described as being both multivariable and multivariate as defined below. These are unique strengths of the method, since they work to increase the signal to noise ratio;

- Multivariable
    - Multiple variables can be combined into one stronger latent variable (**increasing signal**)
- Multivariate
    - Confounding orthogonal variation can be subtracted from variation of interest (**decreasing noise**)

OPLS-DA is often used to discriminate between two sample classes, i.e. cases and controls. In this case OPLS-DA can be seen as a univariate independent t-test. However, studies involving dependent samples are becoming more frequent in metabolomics applications, e.g. samples from the same patient before and after a treatment or individually matched cases and controls. OPLS-DA is not designed for, nor optimal, in such cases, since it does not consider the sample dependence. For this reason, we developed an extension of OPLS, named OPLS-effect projections (OPLS-EP)[2], designed to handle the dependent sample case. OPLS-EP is thus the multivariable and multivariate extension of a univariate dependent t-test. Building on OPLS-EP we have been able to further extend the OPLS framework to also include analysis of intervention studies, short time series and more complex study designs including individually matched cases and controls at different time points.

The main reason why OPLS was developed (from PLS) was improved interpretation of models. However, we believe that there is still room for further improvement in this respect. Thus, we will here also present a strategy for improved interpretation of OPLS models including;

1. Linking statistical significance to OPLS loadings and weights, making interpretation less subjective.
2. Multivariate interpretation of loadings or weights, to increase sensitivity.
3. Combining weights and loadings, to increase accuracy.

Overall we will show that OPLS can be used in different types of studies and that interpretation of OPLS models can provide more detailed descriptions of studied systems, beyond univariate interpretations of individual variables.

(1) Trygg, J.; Wold, S. *J. Chemom.* **2002**, *16*, 119-128.
(2) Jonsson, P.; Wuolikainen, A.; Thysell, E.; Chorell, E.; Stattin, P.; Wikstrom, P.; Antti, H. *Metabolomics* **2015**, *11*, 1667-1678.

Title
Application of Machine Learning Methods to Predict Usage, Age, and Harvest Season in 66 apple cultivars

Authors (presenting author underlined)
Maria Anastasiadi[1], Fady Mohareb[1], Sally P. Redfern[2], Mark Berry[2], Monique S.J. Simmonds[3], Leon

A. Terry[1]

Affiliations
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[3]Royal Botanic Gardens, Kew, Surrey, UK

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Abstract text

The phenolic and sugar profile of a large selection of heritage and modern apple cultivars sourced from the UK's National Fruit Collection was characterised in a three year study including dessert, ornamental, cider and culinary apples. Machine Learning methods were applied with the objective to identify whether the biochemical composition of an apple cultivar could be used as a biomarker fingerprint to differentiate between heritage and mainstream commercial cultivars as well as govern the separation among primary usage groups and harvest season. Prediction accuracy > 90% was achieved with Random Forest for all three models. The high success rates achieved highlight the potential of machine learning for mapping the metabolic profile of a large collection of apple cultivars intended for very diverse uses and introduction dates spanning over several centuries. Knowledge of the key metabolites contributing to flavour and/or health-promoting properties could guide cultivar selection in future breeding programmes on the basis of phytochemical content.

Systems biology in Hepatology: Integration of metabolomics with other omics data

Adil Mardinoglu

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[2]Department of Biology and Biological Engineering, Chalmers University of Technology,

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Abstract text

To develop novel strategies for prevention and treatment as well as to gain detailed insights about the underlying molecular mechanisms of liver associated diseases fatty liver disease, cirrhosis, type 2 diabetes and hepatocellular carcinoma, it is vital to study the biological functions of liver and how liver interacts with other human tissues as well as with the gut microbiota. Biological networks including metabolic, transcriptional regulatory, protein-protein interaction, signalling and co-expression networks can provide a scaffold for studying biological pathways operating in the liver in connection with disease development in a systematic manner. In my presentation, I will present our recent work where biological networks have been employed to identify the reprogramming in liver physiology in response to complex diseases including NASH/NAFLD by integrating metabolomics, transcriptomics, proteomics and metagenomics data. I will further discuss how this mechanistic modelling approach can contribute to the discovery of biomarkers and identification of drug targets which may lead to design of targeted and effective treatment strategies. Finally, I will present a roadmap for the successful integration of models of the liver and other human tissues to simulate whole-body metabolic functions in liver diseases.

(1) A. Mardinoglu, J. Borén, U. Smith, M. Uhlén and J. Nielsen, Systems biology in hepatology: approaches and applications, Nature Reviews Gastroenterology & Hepatology, doi:10.1038/s41575-018-0007-8, 2018.
(2) A. Mardinoglu, A. Hakkarainen, R. Mancina, S. Räsänen, K. Pietiläinen, S. Söderlund, N. Matikainen, E. Björnson, M. Ståhlman, N. Lundbom, H. Marschall, K. J. Williams, S. Romeo, M. Taskinen and J. Borén, An Integrated Understanding of the Rapid Metabolic Benefits of a Carbohydrate-Restricted Diet on Hepatic Steatosis in Humans, Cell Metabolism, doi.org/10.1016/j.cmet.2018.01.005, 2018.
(3) S. Lee, C. Zhang, Z. Liu, M. Klevstig, B. Mukhopadhyay, M. Bergentall, R. Cinar, M. Ståhlman, N. Sikanic, J. K. Park, S. Deshmukh, A. M. Harzandi, T. Kuijpers, M. Grøtli, S. J. Elsässer, B. D. Piening, M. Snyder, U. Smith, J. Nielsen, F. Bäckhed, G. Kunos, M. Uhlen, J. Boren,*, A. Mardinoglu*, Network analyses identify liver-specific targets for treating liver diseases, Molecular Systems Biology, 13:938, 2017.
(4) M. Uhlen, C. Zhang, S. Lee, E. Sjöstedt, L. Fagerberg, G. Bidkhori, R. Benfeitas, M. Arif, K. Sanli, K von Felitzen, P. Oksvold, E. Lundberg, S. Hober, P. Nilsson, J. Mattsson, H. Brunnström, B. Glimelius, T. Sjöblom, P.H. Edqvist, D. Djureinovic, P. Micke, C. Lindskog, A. Mardinoglu# and F. Ponten#, A pathology atlas of the human cancer transcriptome, Science, eaan2507, 2017.

Systems biology of Oral and Gut Microbiome in health and diseases

Saeed Shoaie[1]
[1]Centre for Host-Microbiome Interaction, Dental Institute, King's college London, SE1 9RT, London, UK.

Microbiome for prediction and intervention has rapidly developed itself as the newest and most promising area of biomedical science. The generation of latest gut integrated gene catalogue, with 9.9 million genes has greatly improved the interpretation of gut metagenomics data in association with more than 20 diseases. We generated the first oral integrated gene catalogue with 9.2 million nonredundant bacterial and fungal genes. These state-of-the-art resources allows us to study the function of oral microbial community in gene and species level and integrate the results with the gut microbiome and host for prediction and intervention for impaired liver metabolism. Generating the metagenomics and metabolomics data have shown to be the most effective multi-omics data to understand the mechanisms of disease, early diagnosis and proposing novel intervention using the microbiome compositions and profile of the metabolites. To integrate such a complex and multi-dimensional data on the complex microbiome projects, having saliva and stool samples, requires innovative mathematical formulation and multi-level optimization algorithms. To this end we have generated the first reference model for oral microbiome based on our gene catalog and reconstructed the 800 oral metagenomics species and compared it with the gut metagenome species. This platform made it possible to integrate the metabolomics data on disease cases on saliva and stool samples to further understanding the role of oral and stool microbiome compositions on the progression and mechanisms of microbiome involvement and identification of potential intervention.

Metabolic Systems Analysis of Epithelial to Mesenchymal Cell Transition in Breast Epithelium.

Óttar Rolfsson[1], Qiong Wang, Sigurður T. Karvelsson, Siver Moestu[2] and Skarphéðinn Halldórsson[1].

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[1]Medical School, Centre For Systems Biology, University Of Iceland, Reykjavik, Iceland. [2]Institute for clinical and molecular medicine, NTNU, Trondheim, Norway.

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Epithelial cell to mesenchymal cell transition (EMT) plays a role in cancer progression but is also an important cellular program in healthy embryonic development and wound healing. Metabolism changes during EMT. The significance of metabolism is not understood in the context of altered cell migration and other phenotypic changes that accompany EMT. Employing an omics based approach, we have studied the differences in metabolism in two isogenic breast epithelial and mesenchymal cell lines representative of EMT.

UPLC-MS metabolomics analysis of spent culture medium from these cells in culture revealed changes to glycolysis, the oxidation of glutamine and of branched chain amino acids. These were confirmed with NMR and enzymatic based measurements. Analysis of these data in the context of mRNA expression data or proteomics data using cell specific genome scale metabolic models (GEMs), highlighted changes to lipid metabolism following EMT. A knockdown of ACLY in the epithelial cell line resulted in phenotypic switching to the mesenchymal phenotype. Metabolic modelling however failed to account for how differences in nutrient consumption contribute to fatty acid synthesis. Specifically, discrepancies in TCA cycle flux were observed when metabolomics data were analyzed in the context of mRNA or proteomics data, respectively. 1,2-^{13}C glucose and 1-^{13}C glutamine tracer analysis however showed that the mesenchymal cells are more reliant on the reductive carboxylation of glutamine for citrate production which was further supported by assaying the metabolic effects of the knockdown of IDH2. Finally, changes in lipid content were confirmed using UPLC-MS analysis and showed that changes to fatty acid chain length and saturation along with differences in phosphatidylcholine and plasminogen abundance accompany EMT. This work defines the epithelial and mesenchymal metabolic phenotypes of a selected EMT cell model *in vitro* which we are at various stages of confirming in alternate cell models of EMT in order to identify universal biomarkers of EMT.

Title

Genome-wide metabolic modelling of human CD4+ T-helper cell

Authors

Partho Sen[1], Ubaid Ullah[1], Esko Kemppainen[1], Tanja Buchacher[1], Syed Bilal Ahmad Andrabi[1], Omid Rasool[1], Riitta Lahesmaa[1], Matej Oresic[1,2]

Affiliations

[1]Turku Centre for Biotechnology, University of Turku and Åbo Akademi University, Turku, Finland
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Abstract

T-helper (Th) or T cells play a pivotal role in cell-mediated immunity. Activated CD4+ T cells are mainly divided into Th1, Th2, Th17 and regulatory T-cell (iTreg) subsets, based on the panel of cytokines produced. During the development, T cells undergo metabolic remodeling that is essential for orchestrating the action of other immune cells and for adaptation to the environmental stressors. Moreover, changes in T-cell metabolism have been shown to enhance or suppress their specific functions. In order to understand global metabolism during T-cell development (activation, differentiation), we developed genome-scale metabolic models for human Th1, Th2 and Th17 subsets along with iTreg. Meta-analysis of Th-specific transcriptomics datasets have identified metabolic genes for each subset that are differentially expressed from their naïve controls. The study identified 72 novel metabolic genes, corresponding to 355 reactions spanning various metabolic subsystems in T cells. This genes and reactions were under-reported in generic human metabolic reactions such as in RECON2 and Human Metabolic Atlas (HMR). Therefore, we developed a consensus human metabolic reconstruction named HTimmR of 8539 reactions, 6546 metabolites and 3841 genes. HTimmR model was contextualized with Th-specific transcriptomics datasets and active reactions were filtered. Genome-scale metabolic models were then reconstructed, curated and subjected to further analysis. Reporter metabolites and pathway overrepresentation analysis suggested that T-cell activation induces gluconeogenesis, glutaminolysis, lipid biosynthesis and nucleotide metabolism and subsequently suppress mitochondrial carnitine transport and β-oxidation of fatty acids. These findings are being experimentally validated. Our results suggest that Th subsets exhibit unique metabolic phenotype, already during early stages (72 h) of specification, thus playing a central role in guiding the fate of the cells. The study also points out to specific metabolites and pathways that enhance our knowledge and understanding of immune cell metabolism and its related functions. In addition, the findings provide a basis for modulation of human Th subsets, crucial for immune responses under metabolically aberrant conditions and in immune-mediated disorders.

1st Nordic Metabolomics Conference
Örebro, August 26-28, 2018

ABSTRACT

Title

Metabolome *en route* to autoimmunity and overt disease: prospective studies in type 1 diabetes and celiac disease

Authors
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Abstract text

The metabolome is sensitive to genetic and environmental factors contributing to complex diseases such as type 1 diabetes (T1D) and celiac disease (CD). Our metabolomic studies in T1D and CD suggest that specific metabolic profiles, or metabotypes, precede autoimmunity and overt disease. In both diseases, these early metabolic changes are attributed to many biochemical pathways, thus suggesting disease-specific systemic changes in metabolism which may be inborn or due to complex gene-environment interactions during infancy. Based on this evidence, the role of the metabolome in the progression to these two diseases is therefore to facilitate specific biochemical disease-associated processes, and to contribute to the development of a vulnerable state in which disease is more likely to be triggered. This may have important implications for the understanding of pathophysiology of specific autoimmune diseases and for early disease detection and prevention.

Title
Utility of NMR plasma metabolomics as a diagnostic biomarker in antibody-mediated inflammatory demyelinating diseases of the central nervous system

Authors (presenting author underlined)
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Abstract text
The discovery of autoantibodies against aquaporin-4 (AQP4-Ab) and conformational myelin oligodendrocyte glycoprotein (MOG-Ab) has led to the emerging recognition that these antibody-mediated diseases are pathophysiologically distinct from multiple sclerosis (MS), as well as from each other. Since no pathognomonic clinical features separating these conditions exist, antibody detection remains the cornerstone in diagnosis. However antibody titres can decrease with treatment and after relapses, presenting a diagnostic challenge in patients with borderline/undetectable serum antibody levels. Furthermore, there exists a group of patients negative for both AQP4-Ab and MOG-Ab from the outset (i.e. 'double negative'), with clinical features overlapping MS and antibody-mediated diseases. Conceivably, this 'double negative' group could include patients with atypical MS and antibody-mediated diseases with very low antibody levels. Precise pathophysiological classification of these patients is hugely important as it is recognised that MS drugs exacerbate antibody-mediated diseases. Using nuclear magnetic resonance (NMR) plasma metabolomics, we can reliably distinguish MS from both AQP4-Ab and MOG-Ab diseases, with an accuracy of 94% and 73% respectively. In this study, we explored 1) if the plasma metabolic distinction of these antibody-mediated diseases from MS is independent of antibody titre, and 2) the metabolic-clinical associations of 'double negative' patients. We identified 24 AQP4-Ab disease patients (previously having robust antibody titre, i.e. $\geq$ 200) with low antibody titres i.e. < 200 at the time of plasma metabolomics sampling. Using our established AQP4-Ab disease vs. MS model, 92% of the low antibody patients were accurately classified as AQP4-Ab disease. Indeed, all 4 AQP4-Ab disease patients with undetectable antibody levels were correctly classified. Using the same approach, 2 MOG-Ab patients seronegative at sampling were correctly classified. We then developed a model to discriminate antibody-mediated diseases from MS. This predictive model was able to identify patients with metabolite profiles consistent with either antibody-mediated disease or MS with 82% accuracy. In a cohort of 41 'double negative' patients, 31 were identified as antibody-mediated based on metabolic profiling alone. Comparative analysis using pre-collected clinico-radiological data demonstrated a higher proportion of simultaneous bilateral optic neuritis in these 31 patients, consistent with antibody-mediated disease. The metabolite profiles of the other 10 patients were suggestive of MS. Indeed, more patients in this group were classified as MS-like by 2 neurologists using clinical parameters, before metabolic profiling. These results illustrate the potential of metabolomics to identify patients with antibody-mediated diseases who have undetectable antibody levels, and allow accurate pathophysiological classification in 'double negative' patients.

Human milk metabolomes are shaped by maternal psychological distress and milk cortisol levels

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Human milk is a complex biofluid providing essential nutrients, energy and protection for the infant. The milk composition is subject to considerable inter- and intraindividual variation, manifesting e.g. maternal phenotype and diet. However, the effects of maternal conditions such as stress on the human milk metabolome are not fully understood. Human milk may potentially modulate the transmission of maternal stress conditions to the offspring, thus having a significant effect on the infant's development and stress regulation. Our aim was to apply metabolomics to determine how maternal psychological distress (evaluated by using standardized self-report questionnaires) and milk stress hormone (cortisol) levels are reflected in human milk metabolome, and to reveal the ability of human milk to function as a resilience factor in stress transmission in early life. This work is part of the FinnBrain Birth Cohort Study addressing the effects of early life stress on child development and prospective health (1). Human milk samples ($n = 150$) were collected at 2.5 months postpartum and analyzed with ^{1}H NMR (2). The samples were classified for multivariate analysis based on the following criteria: a) high milk cortisol, b) high prenatal distress (depression and anxiety symptoms) and low milk cortisol, and c) low distress and low milk cortisol (control). Based on the NMR data, 15.5% of the milk samples reflected the maternal fucosyltransferase 2 non-secretor status, which explained 50.8% of the compositional variation in the milks (PCA, t[1]). Untargeted multivariate analyses (OPLS-DA) revealed several metabolites as potential biomarker candidates for maternal stress. For example, fumarate and urea, products of the urea cycle, correlated positively with the high and low cortisol groups, respectively. Hippurate was also associated with low cortisol. The high distress group again was characterized by short-chain fatty acids, associated with microbial metabolism. In conclusion, the data showed that psychological distress and milk cortisol are differentially associated with the human milk metabolome. Among the possible mechanisms are the regulation of the urea cycle and the gut microbiome–host metabolism interaction. Targeted profiling of individual metabolites and their statistical significance with respect to stress, mother and infant-related factors will further clarify the role of human milk in stress exposure in early life.

(1) Karlsson L, et al. Cohort profile: FinnBrain Birth Cohort Study group. Int J Epidemiol. 2018;47(1):15-16j.
(2) Smilowitz JT, et al. The human milk metabolome reveals diverse oligosaccharide profiles. J Nutr. 2013;143:1709-1718.

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ABSTRACT

Title
Bovine colostrum modifies the metabolic response during neonatal bloodstream sepsis in preterm pigs

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Abstract text

Background. Neonatal sepsis, the clinical manifestation of a serious infection, is among the most common complications in preterm infants and increases the risk of brain injury and neurodevelopmental impairment (1). During neonatal sepsis, systemic metabolic adaptations occur, however, the condition of cerebral energy metabolism, which could be of potential importance, is less known. Using a clinically relevant bloodstream infection model in preterm pigs, we previously showed that oral bovine colostrum (BC) supplementation reduced sepsis incidence and brain inflammation. In the current study, we hypothesized that neonatal bloodstream infection would diet-dependently change the metabolomes of the plasma, cerebrospinal fluid and brain tissue. **Methods**. Twenty-six cesarean-delivered preterm pigs received 10^9 CFU/kg *Staphylococcus epidermidis* (SE) systemically and were fed either BC (n=10) or total parenteral nutrition (TPN) (n=9). A control group received systemic saline and TPN (n=7). After 24 h, we collected plasma, cerebrospinal fluid (CSF) and brain tissue for ^{1}H NMR-based metabolomics analyses. **Results**. Multivariate analysis of the NMR data revealed that lactate, alanine and succinate levels, which are involved in anaerobic glycolysis and energy metabolism, were affected by SE infection. Increased plasma lactate, alanine and succinate levels during SE infection (all $p < 0.05$) were confirmed by ANOVA analysis. In CSF and plasma, *myo*-inositol levels were increased in response to SE infection, which might be a marker of an altered glucose metabolism. Higher levels of lactate in the septic brain tissues were found, suggesting a neurodevelopmental impairment caused by the sepsis condition. Intriguingly, BC supplementation reduced plasma and CSF lactate levels in SE infected animals, suggesting that BC may protect the preterm brain against hypoxia-induced brain injury. **Conclusion**. In preterm newborn pigs, hypoxia-associated changes in systemic and cerebral energy metabolism are reduced by oral BC supplementation. *Myo*-inositol, a glucose derivative known for its beneficial effects on lung maturation of preterms, is increased following bloodstream infection and may improve pulmonary function during sepsis.

(1) Brunse, A., et al. Oral Supplementation with Bovine Colostrum Prevents Septic Shock and Brain Barrier Disruption During Bloodstream Infection in Preterm Newborn Pigs. Shock (Augusta, Ga.) 2018.

Title
Metabolomics in nutrition - current status and future outlook

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Abstract text

Applications of metabolomics in nutrition research has increased in recent years. The applications can be generally be grouped into one of the following: (1) Applications to identify dietary biomarkers for single foods or for dietary patterns (2) Applications to dietary intervention studies to help understand metabolic alterations following certain diets and (3) Applications to diet-related diseases. With respect to dietary biomarkers there has been a proliferation of publications in this field: these biomarkers have the potential to act as objective measures of dietary intake thus overcoming some of the key issues with traditional assessment methods. To date, metabolomic profiling has been successful in identifying a number of putative biomarkers of food intake. A number of approaches can be taken when identifying new biomarkers via metabolomics: acute feeding studies with multiple collection of biofluids or association studies in cross-sectional studies. Either way, a key aspect for confirmation of the biomarker is a dose response study. Recently, we used an acute study design where participants consumed standardized breakfasts for three consecutive days over three weeks [1]: the quantity of the food of interest was varied over the weeks. In this instance, intake of orange juice decreased over a 3 week period from average of 520 g/day to 30 g/day. Calibration curves were constructed with the urinary proline betaine concentration against the known orange juice intake (g/day). A correlation of 0.92 was reported between actual intake and predicted intake highlighting the high level of agreement. Importantly, we then applied these calibration curves to biomarker measurement in a cross-sectional study and estimated citrus intake. Good agreement with the self-reported data indicated that this approach could be used in large epidemiology studies to estimate intake. Similarly, use of combination of biomarkers can be employed to study dietary patterns. While significant progress has been made in this field a number of challenges remain and will be discussed.

1.   Gibbons, H., et al., Demonstration of the utility of biomarkers for dietary intake assessment; proline betaine as an example. Mol Nutr Food Res, 2017.

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Title
Biomarkers of fish intake and their association with type 2 diabetes risk

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Abstract

Background: Conflicting evidence exists regarding the association between consumption of fish with incidence of type 2 diabetes (T2D) (1–3). Measurement errors inherent in self-reported dietary assessment methods and co-exposure to persistent organic pollutants (POPs) present in fish may contribute to such inconsistence(3–5). Investigation of associations between plasma metabolites associated with fish intake, POPs and future T2D, may provide new insights into the impact of fish in T2D prevention.
Objective: We aimed to identify metabolite biomarkers of fish intake, assess their associations with T2D risk, and to investigate whether associations could be affected by POP exposures.
Methods: In a Swedish population-based prospective cohort, plasma samples from 503 case-control pairs at baseline and 10-year follow-up samples from a subset of 187 pairs were analyzed using untargeted LC-MS metabolomics. Plasma concentrations of 21 POPs were quantified from the subset with follow-up samples. Fish-related metabolites were identified by supervised multivariate analysis, followed by partial correlation analysis adjusting for confounders. Principal component analysis (PCA) was performed to derive fish-related metabolite patterns. Correlations between fish intake, fish-related metabolites and POPs were assessed by canonical correlation. Odds ratios (OR) of T2D for metabolites and POPs were estimated using conditional logistic regression. Long term reproducibility of metabolites was assessed by intra-class correlation (ICC) in the subset of healthy controls.
Results: We found 67 metabolites associated with fish intake measured by food frequency questionnaires (-0.19 ≤r 0.29, 0.1≤ICC≤0.5). Two PCs accounted for 68% of their total variability were determined for those metabolites, of which PC1 showed strongest association with fish intake and had high reproducibility over 10 years, but was not related with T2D risk. Among metabolites with high PC1 loadings, 11 were individually associated with increased risk of T2D while 7 were associated with decreased risk. Moreover, 16 POPs were positively correlated with fish intake and fish-related metabolites, and were also positively associated with T2D risk. Adjusting risk models for POPs exposures substantially lowered odds ratios for all fish-related metabolites, resulting in a negative association between PC1 and future T2D.
Conclusions: No association between fish intake and T2D risk was observed in the current population. However, several fish-related metabolites were associated with T2D risk in different directions, when assessed individually, suggesting different roles in relation to T2D development. Co-exposure to POPs present in fish may cancel out any potential protective effect of fish intake on T2D risk.

References
(1) Patel PS, et al. The prospective association between total and type of fish intake and type 2 diabetes in 8 European countries: EPIC-InterAct study. Am J Clin Nutr. 2012,95(6),1445–53.
(2] Schwingshackl L, et al. Food groups and risk of type 2 diabetes mellitus: a systematic review and meta-analysis of prospective studies. Eur J Epidemiol. 2017,32(5),363–75.

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ABSTRACT

Title
Metabolic profiling of high egg consumption and the associated lower risk of type 2 diabetes in middle-aged Finnish men

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Abstract text

High egg intake has traditionally been discouraged because the high cholesterol content may impair glucose metabolism and promote inflammation, thereby potentially increasing the risk of type 2 diabetes (T2D). However, in middle-aged men from the prospective, population-based Kuopio Ischaemic Heart Disease Risk Factor Study (KIHD) in eastern Finland, higher egg intake was previously associated with a lower risk of T2D (1). This study aimed to examine potential biomarkers linking egg intake with the lower T2D risk using non-targeted metabolic profiling. We analyzed 239 baseline serum samples from the KIHD in 4 groups: 1) subjects with high egg consumption (mean intake 1 egg/d) who remained free of T2D during the mean follow-up of 19.3 years (controls), 2) controls with low egg intake (about 2 eggs/wk), 3) subjects who developed T2D during the follow-up (cases) with high egg intake, and 4) T2D cases with low egg intake. The non-targeted metabolic profiling employed liquid chromatography coupled with tandem mass spectrometry analysis. Higher egg intake was positively associated with indolelactic acid and two (lyso)phosphatidylcholines ((lyso)PCs) containing 21:2 fatty acid, and negatively with unsaturated acylcarnitines, lysophosphatidylethanolamines, (lyso)PCs, and γ-glutamylated-branched-chain amino acids. Those who developed T2D had higher levels of tyrosine and PCs containing saturated and medium-chain fatty acids, whereas controls had predominantly choline, phosphocholine, glycerophosphocholine, plasmalogens, and (lyso)PCs containing odd- and long-chain polyunsaturated fatty acids. In this study population, we distinguished the metabolic profiles of subjects with varying egg intake and incidence of T2D, with some metabolic overlap on the compounds involved in metabolisms of (phospho)lipids and amino acids. These findings may imply the potential of those metabolites in mediating the previously observed inverse association between egg intake and T2D risk.

(1) Virtanen JK, et al. Egg consumption and risk of incident type 2 diabetes in men: the Kuopio Ischaemic Heart Disease Risk Factor Study. Am J Clin Nutr 2015 Apr 1;101:1088-96.

Title

Gender differences in postprandial response to different dairy products on lipoprotein subclasses: a randomized controlled cross-over trial

Authors (presenting author underlined)

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Abstract text

Background: Measuring lipoprotein subclasses may enable better cardiovascular risk estimations from blood lipid profiles. Dairy products differ in nutrient content and food matrix, and little is known about how different dairy products affect the postprandial levels of lipoprotein subclasses. **Objective:** To investigate how intake of isocaloric high-fat meals with the same amount of fat, but from different dairy products, affect postprandial lipoprotein subclasses in women and men respectively. **Methods:** Thirty-three women (median body mass index (BMI) 21.8 kg/m^2) and 14 men (median BMI 24.2 kg/m^2) were recruited to a randomized controlled cross-over study with four dairy meals consisting of three slices of white bread with jam and either butter, cheese, whipped cream or sour cream, corresponding to 45 grams (approximately 60 E%) of fat. Blood samples were taken fasting and at 2, 4 and 6 hours after ingesting the meals. Lipoprotein subclasses were measured using nuclear magnetic resonance (NMR) for comprehensive profiling of blood metabolites. The incremental area under the curve (iAUC) was calculated for the lipoprotein subclass particle concentrations, and data were analyzed using a linear mixed model. **Results:** Overall, women showed a trend towards smaller increases of the major VLDL subclasses and larger increases of the major HDL subclasses than men. In women, cheese significantly induced the largest increase of XXL-, XL-, L-, and M-VLDL. On the contrary, cheese decreased IDL, L-, M-, and S-LDL significantly compared to whipped cream. Sour cream significantly induced the largest increase of XL-, L-, and M-HDL. In men, S-VLDL increased significantly less after intake of sour cream compared to butter and whipped cream, and XS-VLDL decreased significantly compared to all other meals. Also IDL decreased significantly compared to intake of butter. **Conclusions:** Intake of isocaloric meals with the same amount of fat from different dairy products induces different postprandial effects on lipoprotein subclass concentrations. Women seem to respond more beneficially to high-fat dairy meals than men.

Title

Metabolomics as a tool to elucidate the effect of inulin fortification of processed meat – a rat intervention study

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Abstract text

Red and processed meat intake has been associated with a possible increased risk of colorectal cancer. Nevertheless, meat constitutes an important part of human diet contributing important micro- and macronutrients. Modifying meat products in order to develop healthier products with reduced harmful effect on colon homeostasis might combat this challenge. Merging dietary fiber with meat is anticipated to be a conceivable strategy for the development of heathier meats, since dietary fiber consumption has been suggested to protect against colorectal cancer development. The suggested protective effect likely involves formation of short chain fatty acids (SCFA) through colonic fermentation [1]. Therefore, the aim of the present study was to investigate the effect of inulin fortification of a meat product on endogenous metabolic and gut microbial-derived responses. Thirty healthy Sprague-Dawley rats were fed one of three experimental diets; inulin-fortified (5.6 %) frankfurter-like sausage (n=12), frankfurter-like sausage without fortification (n=12) or a standard chow diet (n=6). Metabolomics approaches using ^{1}H NMR spectroscopic analysis of feces and plasma combined with 16S rRNA gene amplicon sequencing for analysis of gut microbial composition were used. The metabolomics data revealed pronounced effects of diet on the plasma and in particular the fecal metabolome. Levels of SCFAs increased after consumption of the inulin-fortified frankfurter compared to the frankfurter without fortification. These SCFAs are most likely metabolic products of colonic fermentations and the effect of diet is likely to be a result of a modified gut microbiota. In fact, strong effects of diets on the gut microbial composition were found, and the presence of the SCFA butyrate was found to correlate with *Clostridium,* a genus known to comprise butyrate-producing species [2]. In addition, rats fed the inulin-fortified sausages had increased fecal levels of *Bifidobacterium* as compared with rats fed the conventional-like sausages. The findings reveal that inulin fortification of processed meats resembles the general effect seen upon dietary fiber consumption. In conclusion, the study corroborates that the introduction of fiber fortification strategies in the manufacturing of meat products could have a promising potential to combat possible harmful meat-induced effects in the colon.

1. Bingham SA, et al. Dietary fibre in food and protection against colorectal cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC): an observational study. *Lancet (London, England)*. **2003**;361(9368):1496-501.
2. Pryde SE, et al. The microbiology of butyrate formation in the human colon. *FEMS microbiology letters*. **2002**;217(2):133-9.

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Title

Investigation of Pyrazinamide Mechanism of Action for Tuberculosis Using Metabolomics

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Abstract text

Tuberculosis (TB) is both the leading cause of deaths due to an infectious disease and the leading cause of deaths due to a curable disease[1]. However, drug resistance is increasing while the pipeline of new drugs stagnates, and knowledge of existing drugs remains incomplete. Pyrazinamide (PZA) is a frontline TB drug whose mechanism of action remains among the most poorly understood.

Here, we present a high-performance ion-pairing reversed-phase (IP-RP) Q-TOF LC/MS method that has enabled the biologically unbiased study of the impact of PZA on the Mycobacterium tuberculosis metabolome. Coupled with batch feature extraction and multivariate statistical analysis software, this workflow enabled the discovery of activity-specific metabolic changes that may help explain PZA's unique metabolic effects.

A non-targeted metabolomic study of retail pomegranate juice products to investigate nutritional and quality characteristics - using a novel data independent acquisition mode and ion-mobility on a QTof MS instrument

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Comprehensive identification of the phytoactive compounds is a critical starting point for assessing the biological and technological properties in food matrices. Due to the complexity of plant secondary metabolism the full characterisation of phytochemicals in fruits and vegetables is recognised as a significant analytical challenge and requires sensitive and accurate techniques to be employed. Pomegranate fruit (*Punica granatum* L.) is commonly reported as a rich dietary source of phenolic compounds with regular consumption being linked to a wide range of associated health benefits. Phenolic compounds are also known to play an important role in the quality and sensorial performance of fruit juice products and as such of value to the food industry.

The diverse array of different (poly)phenolic structures including flavonoids, phenolic acids and hydrolysable tannins can make the accurate screening of their profile difficult. High resolution chromatographic techniques, coupled with Quadrupole Time of Flight (UPLC-QToF) MS detection can provide the information to achieve this. However, the resulting data output often consists of retention time, precursor ion exact mass, fragment ion(s) exact mass, isotopic profile and intensity data for every component in each sample. In order to rationalise the results and identify compounds of significance to the food industry, multivariate statistical approaches including principal components analysis (PCA) and orthogonal partial least squares discriminate analysis (OPLS-DA) are commonly used approaches to elucidate population variations and interpret these complicated data sets.

In this study, we report the potential of a new data independent acquisition (DIA) mode on a QTof instrument in combination with a scanning quadrupole mass filter and ultra fast detection system. This methodology alongside ion mobility enabled QTof-MS (IM-QTof-MS) were used as tools to improve analytical selectivity and facilitate the process of marker identification in complex juice samples following a simple sample preparation step. The resulting information was further subject to database searching which indicates the presence of several significant polyphenolic compounds and processing additives in a selection of commercially available processed juice products in the U.K.

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Title
Improved metabolome coverage and increased confidence in unknown identification through novel automated acquisition strategy combining sequential injections and MSn

Authors

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Abstract text

Metabolite identification is a current bottleneck in the broad implementation of metabolomics, hindering biological interpretation of results. The development of fastscanning high resolution accurate mass spectrometers has increased the number of detected metabolites in biological samples. However, many metabolites remain unidentified because of sample complexity and limitations in data-dependent acquisition (DDA). Dataindependent acquisition (DIA) approaches can provide fragmentation for all precursor ions simultaneously, but result in complicated fragmentation spectra, further convoluting the identification process. Here, we describe a data-informed workflow that maximizes the number of metabolites interrogated by MS/MS and MSn, while minimizing the acquisition of uninformative spectra. This innovative workflow was used to analyze human plasma resulting in high confidence identifications, deeper metabolome coverage and enhanced biological knowledge generation. We developed an improved workflow that allows for the direct interrogation of all detected metabolites and applied it to the analysis of human plasma. During data-dependent MS/MS, ions are selected based on abundance, without any knowledge of biological relevance or type of ion. Often, irrelevant spectra, resulting from fragmentation of solvent, plasticizer and other background ions dominate the duty cycle, limiting the capacity of the instrument to acquire informative spectra. In a typical DDA experiment, we determined, that >40% of MS/MS spectra could be attributed to background ions. By enabling the automatic generation and implementation of a background exclusion list based on real-time feature detection in LC-MS data, background ion MS2 spectra were practically eliminated (<0.1%), allowing for the analysis of more true sample components. Small molecules form different types of adducts and cluster ions during electrospray ionization. Highly abundant compounds are represented by many ions and may prevent the fragmentation of lower abundance metabolites. By populating the inclusion list with the preferred ion for each metabolite, more compounds can be sampled by MS/MS in a single run. Additionally, by automatically updating inter-run inclusion and exclusion lists, the subsequent run will prioritize compounds not previously selected for MS/MS. This automated acquisition strategy was implemented on a Tribrid™ mass spectrometer, enabling multistage fragmentation (MSn). The combination of MSn and automatically generated inter-run inclusion and exclusion lists resulted in fragmentation of more unique metabolites and a greater number of metabolites confidently annotated. Application of this innovative workflow addresses the identification bottleneck of untargeted metabolomics studies and enables confident biological interpretation of the results.

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Title
Novel and innovative Solutions for advancing Metabolomics and Lipidomics research

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Abstract text

Temporal and spatial changes in the identity and levels of thousands of metabolites reflect the final outcome (phenotype) of interactions at the genomic, transcriptomic and proteomic levels. The aim of discovery metabolomics is the global profiling of small molecule as well as lipid biomarkers characteristic for particular physiological states which change in response to internal or external perturbations.

Several bottlenecks were described in this rapidly emerging research field, these concern e.g. the identification of unknown compounds, rapid and confident identification of analytes which are already known (called de-replication), as well as sample throughput for clinical and translational research.

This presentation will highlight novel solutions developed by Bruker in collaboration with well-known metabolomics research laboratories to address the above-mentioned challenges. The presentation will highlight MRMS aXcelerate to increase sample throughput with LC free analyses by Flow Injection Analysis - Magnetic Resonance Mass spectrometry (FIA-MRMS). This direct sample analysis workflow driven by extreme resolution enables to analyze over 200 samples a day which is complementary to established NMR based solutions. MRMS aXcelerate can also access compounds not readily detectable by LCMS and the extreme mass resolution and accuracy of MRMS technology allows for 3-tier confidence in formula ID assignments.

An overview of new tools for NMR based Metabolomics in clinical and translational research will be given. Quantification of small molecules in plasma/serum gives access to disease relevant metabolites with high accuracy. In urine besides the 150 metabolites quantified automatically, now also 11 ions can be quantified, which also are related to frequent diseases like high blood pressure or cancer.

There is a strong growth in biobanks as central or hospital owned repositories for human specimens. Standardization is needed throughout biobanks across the world for highest efficiency. NMR standardization can help to perform QC on specimens before input into the biobank and can at the same time deliver standardized spectra and quantitative analysis to be added to the metadata in the biobank. This enables biobank data to be integrated into worldwide clinical trials or epidemiological research.

1st Nordic Metabolomics Conference
Örebro, August 26-28, 2018

ABSTRACT (template)

Title
Metabolomics of Neonatal Blood Spots Reveals Lipids Associated with Childhood Leukemia

Authors (presenting author underlined)
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Abstract text

Early life exposures are believed to greatly influence the incidence of acute lymphoblastic leukemia (ALL), which is the most common form of childhood leukemia. Yet, the causes and underlying mechanisms of ALL initiation remain elusive. Archived neonatal blood spots (NBS, also called Guthrie cards) from newborns offer a means to investigate the fetal blood exposome and its connections with childhood leukemia. We obtained NBS for 334 ALL case-control pairs from the California Birth Registry (Sacramento, CA, USA). Matching of cases and controls was performed on date of birth and gender. NBS punches (4.7-mm), representing approximately 8 microliters of blood, were extracted with acetonitrile and untargeted metabolomics was performed with an Agilent 1290 UHPLC system connected to a 6550 QTOF HRMS (Santa Clara, USA) in ESI (-) mode as described previously[1]. Blank punches from adjacent portions of the same Guthrie cards were used for background adjustment. Data were cleaned and normalized, focusing on common features that were clearly distinguished from blanks and present in most of the samples.

We evaluated 730 common small-molecule features. Analysis was performed on subjects stratified by age at diagnosis as a means of distinguishing between potentially different ALL phenotypes (early: 1-5 years; late: 6-14 years). Covariates representing known or suspected risk factors for ALL were also evaluated. In order to minimize the number of false positives, associations between metabolite features and ALL were determined with an ensemble of statistical methods, namely, linear regression *p*-values, bootstrapped regularized logistic regression (LASSO), and random forest. There was no overlap in features selected for associations with early diagnosis (9 features) and late diagnosis (24 features) of ALL. Selected features were annotated by comparing mass and MS2 fragmentation spectra against publicly available databases, as facilitated by the CompMS2Miner package[2]. Annotated metabolites were primarily fatty acids and glycerophospholipids. Two of the selected metabolites were associated with the time to ALL diagnosis, suggesting that they reflect effects of disease progression rather than causal exposures. Interestingly, several metabolites selected in the late diagnosis group were highly associated with breastfeeding practice - a known protective factor for ALL - and were present at higher levels in ALL cases than controls. (Since NBS are collected within 48 hours of birth they include nutrients from one or more infant feedings). These results suggest that lipids associated with neonatal nutrition may be involved with initiation of ALL in early life and should be a focus of future targeted research.

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Exposure to environmental chemicals during pregnancy affects the lipidomic profiles in the offspring and may mediate the risk of type 1 diabetes

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Exposure to environmental pollutants during fetal or early life has been associated with several adverse health effects, such as adverse fetal growth and with developmental neurotoxic, immunological, and obesogenic effects in offspring.

We combined metabolomics and comprehensive screening of environmental pollutants in order to identify effect-based markers of exposure during fetal development. We studied a well characterized mother-child cohort, with serum samples available both from mothers during the pregnancy and from children at birth and at 3, 6 and 12 months of age. We characterized environmental contaminants from the maternal samples in order to assess the exposure during the fetal development using multiple LC-QqQMS and GC- HRMS methods. We determined the levels of perfluorinated compounds, flame retardants, PCBs and pesticides, and related persistent organic compounds. Additionally, we performed comprehensive metabolic profiling of the whole cohort, using UHPLC-QTOFMS and GC-QTOFMS methods.

We observed significant changes in the lipidomic profiles of the children whose mothers had high levels of perfluronaed compounds during the pregnancy. Specific phospholipids were downregulated, with the pattern of changes similar to the lipid profiles found associated with progression to T1D (1,2). Among the top 12 ranked lipids based on odds of progression to T1D, including the most abundant sphingomyelins and phosphatidylcholines, eight lipids were identical as observed significantly altered (p, FDR q < 0.05) between the low and high exposure during the pregnancy.

In summary, this study suggest that maternal exposure to specific environmental toxicants affects the circulating lipidomic profiles in the offspring, which may, in turn, increase the risk of type 1 diabetes.

References:
1. M. Orešič et al. Diabetes 2013;62: 3268-3274.
2 . D. La Torre et al. , Diabetes 2013, 62: 3951-3956

Metabolite characterization along wood forming tissues in hybrid aspen

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Abstract text

Wood (secondary xylem) is a valuable resource for the generation of renewable energy and it is an important feedstock for fiber, pulp and cellulose production. For industry both wood formation and wood quality are of great importance. Wood formation is a dynamic and continuous process, strongly affected by environmental conditions such as water, nutrient and light availability [1]. Wood formation is a sequential developmental process, including differentiation of vascular cambium cells into secondary xylem mother cells, cell expansion, deposition of secondary walls, programmed cell death, and finally formation of heartwood [2]. Transcription is highly controlled in the wood forming tissue and transcriptomics analyses in tree species have enhanced our understanding of the molecular mechanisms underlying cambial growth and wood formation. However, there is still a lack of information about the metabolite gradient along different cell types in the cambial zone. The analysis of individual tissues eliminates the dilution effect due to complex tissue mixtures, and allows the discovery of very small differences among distinct cell types [3]. A metabolomic approach (targeted and untargeted) was performed to analyse the stem tissues from a four-month-old hybrid aspen (*Populus tremula x P. tremuloides*) trees, cultivated under greenhouse conditions and submitted to different levels of fertilization (adequate, high, low and water). The stems were 20 μm thick tangential cryo-sectioned in: bark, inner bark, phloem, expanding phloem, cambium, expanding xylem, xylem and pith. By multivariate analysis we could discriminate tissues and treatments. A gradient of metabolites was identified along the differentiated wood tissues, e.g. glucose-6-phosphate, fructose-6-phosphate, maltitol, maltose, 2-amino-adipic acid, oleamide and succinic acid were accumulated in cambium and expanding zones. Regarding to the treatments, arginine, asparagine, aspartic acid, glutamine and glutamic acid accumulated under high fertilization. Furthermore, trees submitted to high amount of fertilizer showed high diameter and biomass, compared to low and water treatments. The results are the basis for future studies of how each tissue respond to identify key metabolites related to secondary growth and wood formation in hybrid aspen.

(1) Plavcová L, et al. Linking irradiance induced changes in pit membrane ultrastructure with xylem vulnerability to cavitation. Plant, Cell & Environment. 2011 Mar 34(3):501–513.
(2) Ye ZH et al. Molecular control of wood formation in trees. Journal of Experimental Botany. 2015 Jul 30:66(14) 4119-4131.
(3) Kehr J. Single cell technology. Current Opinion in Plant Biology. 2003 Dec 6(6): 617–621.

Title
The biosynthetic origin of psychoactive kavalactones in kava

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Abstract text

Plants have traditionally served as an important source of medicinal compounds, but their application often requires laborious isolation from the native plant species. Recent advances in genomics, metabolomics, and synthetic biology are increasingly enabling the translation of such ancient medicinal wisdom into modern pharmaceuticals. Kavalactones are the principal psychoactive polyketide natural products found in the rhizome of kava (*Piper methysticum*), a tropical medicinal plant endemic to the Polynesian islands with well-established anxiolytic and analgesic properties. Here we report de novo elucidation of kavalactone biosynthetic pathway consisting of seven specialized metabolic enzymes. Based on phylogenetic and crystallographic analyses, we highlight the parallel gains of two styrylpyrone synthases in kava, both of which have independently neofunctionalized from an ancestral chalcone synthase to catalyze the formation of the kavalactone scaffold. Subsequent enzymatic steps further facilitate regio- and stereo-specific diversification of kavalactones. We demonstrate the feasibility of engineering heterologous production of kavalactones and their derivatives in bacterial, yeast and plant hosts, thus opening an avenue towards the development of novel psychiatric therapeutics.

POSTERS

Metabolomics workflows, data processing, metabolite identification, quality control

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Title
Advanced biomarker discovery through investigation of gut microbiota and human host co-metabolism - Linking metabolomics with chemical biology methodologies

Authors (presenting author underlined)
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Abstract text

The detailed investigation of metabolites in human samples (serum, plasma, urine, saliva, feces or tissues), termed metabolomics, carries a great potential for the discovery of unknown biomarkers. This is urgently required for diseases with advanced stage prognosis such as pancreatic cancer, one of the most lethal cancers worldwide with steadily increasing patient numbers. However, this is a challenging task as biological samples are comprised of a complex mixture of biomolecules. Advanced chemical biology tools are limited for this newest 'omics'-research field.

One of the most exciting scientific developments in the past decade has been the understanding that gut microbiota profoundly impact human physiology.[1,2] The complex consortium of trillions of microbes possesses a wide range of metabolic activity. This metabolic interspecies communication represents a tremendous opportunity for biomarker discovery as only limited information on this co-metabolism has been elucidated on a molecular level.[3]

We have developed new chemical biology techniques for an enhanced metabolomics analysis using liquid chromatography-coupled with tandem mass spectrometry (UPLC-MS/MS). Our research is based on selective biochemical conversion of metabolites for qualitative and quantitative analysis through chemical synthesis of (isotope-labeled) reference molecules. We have combined these new methods with state-of-the-art mass spectrometric and bioinformatics techniques to enhance the scope of general metabolomics studies. Our approach represents a new and unique strategy for metabolite analysis and is focused on host-microbiota co-metabolism.

The detailed investigation of small molecule metabolites in human samples including feces, serum/plasma and urine, represents a high potential for discovery of unknown biomarkers. Application of our new metabolite-analyzing methodologies at the interface of chemistry and biology led to the identification of a series of previously unknown metabolites using mass spectrometry-based metabolomics.

(1) Turnbaugh, P. J. *et al.* A core gut microbiome in obese and lean twins. *Nature* **2009,** *457* (7228), 480-4.
(2) Liu, R. *et al.* Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. *Nat. Med.* **2017,** *23* (7), 859-868.
(3) Wikoff, W. R *et al* Metabolomics analysis reveals large effects of gut microflora on mammalian blood metabolites. *Proc. Natl. Acad. Sci. U. S. A.* **2009,** *106* (10), 3698-3703.

Title

Construction and application of a high-resolution MS/MS library including retention time information for rapid identification of endogenous metabolites

Authors (presenting author <u>underlined</u>)

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Abstract text

High-resolution LC-MS is an important platform for metabolite detection and quantitation. However, for untargeted Metabolomics rapid, unambiguous and universal compound identification is still challenging. In this work, we report the construction of a library for relevant endogenous metabolite and its' successful application to human biofluids.

Standards were obtained from the Human Metabolome Database (HMDB). They were injected into an Intensity Solo C18 column via an Elute LC system and detected by a QTOF-MS (impact II, Bruker Daltonics) for acquiring MS/MS library spectra and the retention time determination (RT).
For the analysis of biofluids the same setup was used following a standard operating protocol (SOP) for consistency. Automatic metabolite identification was performed in the MetaboScape software based on matching of multiple parameters: precursor mass accuracy and isotopic pattern, RT, and MS/MS spectrum quality.

In this study a library from over 800 endogenous metabolites was created. It contains MS/MS spectra of 635 compounds acquired in positive mode and 474 negative mode spectra. Up to 5 collision energy levels were applied for each standard giving more than 6000 MS/MS spectra in total. For each metabolite, library fragment spectra were manually curated by confirming each fragment via a molecular formula. For unambiguous identification we determined the RT of each standard. An RT correction method using RT standards was applied to balance effects caused by variations in experimental conditions.
Finally, we have examined the portability of this library for different labs. Biofluids such as human urine were analyzed in both positive and negative ion modes in LC-MS, followed by metabolite identification using the library. The comparative results will be presented.

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ABSTRACT

Title
Profiling unknown compounds related to coffee roasting

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Abstract text

Coffee is the second most traded commodity worldwide thus the main contributor of phytochemicals in the diet. Previously, our group reported that the degree of roasting correlates with the efficiency of dampening inflammation and enhancing the antioxidant defence. Therefore, our primary objective was to identify those features that differentiate among coffee with several roasting levels.
Robusta and Arabica coffee beans were roasted in a cylinder roaster at 220°C as follows: light, 6 min; medium, 9 min; and dark, 15 min. Coffee beans were pulverised, and 10 g of the sample was added to 20 mL of boiling-water and 20 mL of methanol. 50 µL of the sample were diluted in 950 µL of water and centrifuged at 3000 g for 10 minutes. The analysis was performed on a platform using an Ultimate 3000 RSLC Quaternary and a Thermo-Fisher Scientific Orbitrap-Q-Exactive. Samples were measured in triplicate in positive and negative mode. Compound Discoverer 2.1 was used to process the raw data and generate a table with relative concentrations. Processed data was Pareto scaled and log-transformed for multivariate analysis, including OPLS-DA and SUS-plotting, in SIMCA15.
A SUS-plot was constructed using the OPLS-DA models from the Light vs Medium or Dark roasting comparisons to identify the compounds specific to each type of roasting. Nine compounds in positive mode and two in negative were related to the medium roasting. Fifteen in positive and two in negative were associated with the dark roasting.
An OPLS-DA modelling accompanied by a SUS-plotting seems to be a practical approach to identify specific-features related to the level of coffee roasting. Further identification of the specific compounds is needed. The relationship between these features and its role in the anti-inflammatory properties of coffee need to be confirmed.

1.   Paur, I., Balstad, T. R. & Blomhoff, R. Degree of roasting is the main determinant of the effects of coffee on NF-kappaB and EpRE. *Free Radic. Biol. Med.* **48,** 1218–27 (2010).

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Title
Experimental considerations for cell secretome-based metabolomics investigations

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Abstract text

The application of metabolomics to investigating the cell secretome has garnered popularity owing to the method's large-scale data output, biochemical insight, and prospects for novel target compound discovery. However, there are no standardized protocols for the use of cell growth media, a factor that can exert profound effects upon the detected metabolites, and thus in the interpretability of the resulting data. Herein, we applied a liquid chromatography-high resolution mass spectrometry-based metabolomics approach to examine the influence of 5 different media combinations upon the obtained secretome of two phenotypically different cell lines: human embryonic kidney cells (HEK293) and L6 rat muscle cells. These media combinations were, M1: Medium 199, M2: Medium 199 + 2% fetal bovine serum (FBS), M3: Dulbecco's Modified Eagle's Medium (DMEM), M4: DMEM + 2% FBS and M5: Krebs-Henseleit Modified Buffer (KHB). The effect of incubation (37 °C) vs. refrigeration (4 °C) on DMEM medium over a 24 h period was also investigated. Results were validated for a selected panel of 5 metabolites measured from an additional cell culture experiment. Metabolomics identified a total of 53 polar metabolites that exhibited differential patterns on a cell type- and medium-specific basis. We observed that choice of media was the primary contributor to the secreted metabolite profile detected. The addition of FBS resulted in unique detected metabolites, compared to media-only controls (M199 and DMEM alone). Glutamine and pyroglutamate were more abundant in incubated relative to refrigerated DMEM medium. The overall metabolic pattern of the metabolites from the targeted approach matched with that exhibited across M1-M5 of the metabolomics experiment, and aided in further identifying the presence of compounds that were below the limit of detection in metabolomics. Based upon these findings, we highlight the following considerations in designing a cell secretome-based metabolite profiling experiment: (1) multiple media combinations (including a range of FBS percentage) should be considered when evaluating a particular cell line; (2) media combinations (i.e., cell-free) should be plated separately, and incubated/treated in the same experimental conditions as the cells. (3) Ideally, a compromise between cell death and optimal detection of metabolites should be evaluated. This approach is necessary to avoid, as much as possible, batch-specific FBS contributions that can render experiments irreproducible. These effects will be dependent upon the metabolites of interest. (4) Finally, the analytical performance of each compound of interest, within the selected cell-culturing conditions, should be evaluated and optimized accordingly to ensure robust outcomes.

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Title
The Turku Metabolomics Centre

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Abstract text

The Turku Metabolomics Centre (TMC) was formed in spring 2017 and officially opened in August 2017. The TMC has expanded its activities across the entire Turku campus, encompassing two Universities and the University Hospital. The aim is to provide world class small molecule analytics for both universities and the wider scientific community, serving both academic and industrial partners. The strength of TMC is the ability to design targeted custom assays for clinical research. The Systems Medicine Group in Turku has both the instruments and the expertise for the development of custom assays for both polar and non-polar compounds. The inclusion of the Bioanalytical Laboratory enables TMC to develop and provide assays in a good laboratory practice (GLP) quality framework. This enables the small molecule assays to be utilised in clinical settings under the requisite regulatory frameworks. Additionally, the inclusion of the Food Chemistry and Food Development Group brings in expertise on both targeted and non-targeted analysis of small molecules in foodstuffs and how the latter impact the overall metabolism of humans and how this affects health. The Food Metabolomics approach offers important tools for food development and food authentication. TMC has access to NMR instruments located at the joint Instrument Centre of the two universities which has the unique capability in Finland to measure metabolites from tissues or cells using 1H-NMR spectroscopy without first extracting the metabolites, achieved by using magic angle spinning technology. The in-vitro metabolic health of such cell cultures can also be monitored using locally available Seahorse technology. This far-reaching network within Turku brings together world class researchers in the field of metabolomics and small molecule analytics, allowing closer collaboration between the groups.

A pneumatically assisted nanospray desorption ionization source for enhanced metabolite imaging from tissue

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Nano-DESI is an ambient liquid extraction technique which enables quantitative MS imaging of metabolite and lipid species. The probe is comprised of two fused silica capillaries separated by a liquid bridge into which analytes are desorbed for subsequent electrospray ionization. Here, we describe the addition of a nebulizer to drive solvent through the secondary capillary to enhance desolvation of small molecules during the ionization process.

To evaluate the device, ion images were generated from rat kidney sections using nano-DESI coupled to a high resolution mass spectrometer with and without a nebulizer. The obtained data demonstrates a 10-1000 times higher S/N for metabolites (m/z 100-300) with the nebulizer. Although the absolute intensities of many metabolites are greater with the nebulizer setup, much of the increased S/N appears to come from a reduction in noise – likely resulting from a more consistent solvent flow and more complete desolvation during the ESI process. Consequently, metabolites (*e.g.,* amino acids) show more dynamic changes when the nebulizer is used, making it possible to observe more intricate biological variance of metabolite abundance within the tissue.

The conventional nano-DESI device requires a significant proportion of organic solvent to enable flow through the secondary capillary. Using the pneumatically assisted nano-DESI device, we report the first liquid extraction MS images obtained without any organic solvents. By use of pure water for extraction polar metabolite species were highly abundant in the acquired mass spectra while lipid species, having low water solubility, were not observed. Use of pure water as a nano-DESI solvent revealed 46 new ion images of polar metabolite species not observable with methanol as the nano-DESI solvent, indicating that this new development will significantly advance imaging of polar metabolites in tissue sections.

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ABSTRACT

Title
Ultrafast detection of drugs and metabolites in urine by Flow Injection Analysis (FIA) coupled to Magnetic Resonance Mass Spectrometry (MRMS)

Authors
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Abstract text

Introduction:
Detection of metabolites and drugs in body fluids such as plasma or urine by LCMS is a routine method in metabolomics and doping analysis. Routine UPLC-MS measurements are performed typically in 15 min. Therefore, the number of analyzed samples is highly limited. In this work, a fast method for detection of drugs and their metabolites in urine using FIA-MRMS is presented. Roughly 250 samples can be measured in 24h using this technique.

Methods:
Six pooled urine samples were purified by SPE using Merck LiChrolutEN SPE cartridges. Samples were extracted with methanol from SPE cartridges and diluted 1 to 100 for FIA. Each samples was analyzed in 5 minutes by FIA-MRMS using a solariX 2xR (Bruker Daltonik, Bremen) in ESI using positive and negative ion mode. Analysis of data was performed with MetaboScape 3.0 (Bruker Daltonik, Bremen).

Results:
The data of the ESI(+) and ESI(-) were combined for feature analysis. More than 2100 features have been found for the pooled urine samples. More than 90% of the detected features could be assigned with a molecular formula. 300 drug candidates have been detected in the urine samples using a HMDB urine database with a mass error tolerance of only 0.5 ppm. The detected drugs have been compared with the medication of the patients. Several drugs have been found only in one or a few pooled urine samples. By comparing the relative abundances of features of all samples, possible metabolites of drugs could be identified.

Conclusions:
Drugs and their metabolites can be detected by FIA-MRMS in a few minutes. This workflow is much fast than the conventional workflow using UPLC-MS. This method could even be used for quantification when internal drug standards are added. Due to the complexity of the samples ultra-high mass resolution as well as very accurate mass detection is a prerequisite for this workflow.

Novel Aspect:
FIA-MRMS can be used for fast detection of drugs and metabolites in urine.

Mass spectrometry imaging of metabolites in zebrafish with nanospray desorption electrospray ionization mass spectrometry

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Introduction

The zebrafish *Danio rerio* is a model vertebrate organism which is routinely used in molecular, reproductive, developmental, and chemical toxicity studies. Mass spectrometry imaging (MSI) enables visualization of the spatial distribution of individual biomolecules in tissue as 2D maps without the need for any pretreatment, such as antibodies or fluorescent staining. The acquired 2D maps contain metabolite signatures providing biochemical activity of tissue regions and thereby insights into pathological and biological processes. Herein, we demonstrate the use of nanospray desorption electrospray ionization (nano-DESI) MSI to distinguish metabolic signature in particular areas of interest in thin tissue sections of zebrafish.

Methods

Nano-DESI MSI was carried out using a custom-built sampling apparatus connected to a Q-Exactive Orbitrap™ mass spectrometer. The nano-DESI solvent consisted of 9:1 MeOH/H_2O (v/v) + 0.1% of formic acid and was pumped through the fused silica primary capillary (ID 50 μm, OD 150 μm) at a flow rate of 0.5 μL/min. To increase the spatial resolution oversampling was performed by stepping 50 μm between each line scan along the y-axis. Each ion image was acquired using a set velocity along the x-axis. The velocities varied between 5-20 μm s^{-1} depending on desired pixel size.

Results

Preliminary results show metabolite and lipid localizations to distinct regions in transversal sections of zebrafish. The addition of formic acid to the nano-DESI solvent enhanced the [M+H]$^{+}$ signal for several analytes and minimized cationization on sodium and potassium, therefore lowering the limit of detection. For example, the bioactive 2-arachidonoylglycerol, known to be present in the central nervous system, shows localization to the spinal cord of the zebrafish. Additional metabolites localizing to the region of the spinal cord include hypoxanthine and several carnitine species, tentatively assigned based on accurate mass. Several plasmalogens, diacylglycerols, creatine, and creatinine were also detected with distinct distributions. In conclusion, our results demonstrate that nano-DESI MSI of transversal sections of zebrafish yields novel metabolite data enabling future studies investigating the spatial distribution and importance of metabolite species in developmental studies.

Profiling the metabolism of human cells by deep ^{13}C labeling

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Abstract

Studying metabolic activities in living cells is crucial for understanding human metabolism, but facile methods for profiling metabolic activities in an unbiased, hypothesis-free manner are still lacking. To address this need, we here introduce the deep labeling method, which combines a custom ^{13}C medium with high-resolution mass spectrometry. A proof-of-principle study on human cancer cells demonstrates that deep labeling can identify hundreds of endogenous metabolites as well as active and inactive pathways. For example, protein and nucleic acids were almost exclusively de novo synthesized, while lipids were partly derived from serum; synthesis of cysteine, carnitine and creatine was absent, suggesting metabolic dependencies; and branched-chain keto acids (BCKA) were formed and metabolized to short-chain acylcarnitines, but did not enter the TCA cycle. Remarkably, BCKA could substitute for essential amino acids to support growth. The deep labeling method may prove useful to map metabolic phenotypes across a range of cell types and conditions.

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Title
A Rapid Microbore Metabolomics Profiling (RaMMP) Analytical Platform for Discovery Metabolomics and Lipidomics

Authors (presenting author underlined)
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Abstract text

A suite of rapid metabolomics LC-MS methodologies, for polar, non-polar small molecule and lipid analysis, has been developed delivering high throughput and high reproducibility biofluid analysis.

As global life-styles change we are seeing increasing cases of obesity, diabetes, and mental health issues. Discovery metabolomics, employing unbiased data collection and analysis, offers a valuable and unique insight into the underlying biochemistry of diseases as well as the patients' individual biochemistry "phenotype', diet, health status, age and stress. To deliver this information the analytical data generated is processed via a variety of chemometric modelling and analysis methodologies to deliver the relevant biochemical information.

LC-MS based analysis has become the key technology for metabolomics providing data on polar and non-polar metabolites as well as lipids. However these analysis times are typically in the 15-30 minute range which is not compatible with large cohort clinical and epidemiological studies where sample sizes often are in the 1000's range. To address this challenge we have developed a suite of sub 3 minute LC-MS assays based on microbore LC and accurate mass MS. The assays have been employed for the analysis of several rodent toxicology samples and human breast cancer samples. The data generated has been shown to be reproducible, sensitive and reliable delivering similar "biomarkers" to that obtained with more extensive metabolomic methods.

Title
Metabolomic analysis of platelet concentrates treated with the INTERCEPT blood system

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Abstract text

Introduction
Platelets represent an important part of transfusion medicine on account of their hemostatic role. Platelet concentrates (PCs) serve as supportive treatments for cancer and hemotological malignancies, marrow failure and more. Storage of platelets is limited to 5-7 days due to increasing threat of pathogen growth and the limited lifetime of the platelets themselves. To diminish the threat of pathogen transmission via blood transfusion, pathogen reduction techniques have been developed. Here we investigate the changes in the platelet metabolome upon treatment with the INTERCEPT blood system.

Methods
Buffy coat platelet concentrates where collected from whole blood and split into 2 groups: The treatment group (n = 8) which was treated with the pathogen reduction system, and an untreated control group (n=8). Samples were collected throughout the storage time and the intra- and extracellular fractions where separated. From both fractions metabolites where extracted and measured with UPLC-qTOF in both positive and negative ESI mode. The data was analyzed using the software package XCMS for untargeted analysis and Targetlynx for a targeted analysis. Selected metabolites where further identified by using LC-MS/MS. Furthermore, ^{13}C tracing was used to confirm the activity of metabolic reactions that had previously not been reported in human platelets.

Preliminary Data
Preliminary data indicates a clear impact on the paltelet metabolome resulting from the pathogen reduction system, with antioxidant defenses being seemingly the most prominent. Furthermore, the non-targeted analysis has revealed the existence of metabolites that have not been previously reported in human platelets. ^{13}C tracer analysis has confirmed the production of these metabolites in platelet during storage.

Title

Interlaboratory coverage test on plant food bioactive compounds and their metabolites by mass spectrometry-based untargeted metabolomics

Authors

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Abstract text

Plant-based foods contain thousands of potentially bioactive compounds, further metabolized endogenously and by the gut microbiota, which present interest for human nutrition and health. State-of-the-art analytical methodologies, such as untargeted metabolomics based on high-resolution mass spectrometry, are required for the profiling of these compounds in complex matrices, including plant food materials and biofluids. Initiated by the COST POSITIVe network (Interindividual variation in response to consumption of plant food bioactives and determinants involved), the aim of this study was to compare the analytical coverage of untargeted metabolomics methods independently developed and employed in various European platforms. In total, 56 chemical standards representing the most common classes of bioactive compounds spread over a wide chemical space were selected and analyzed by the participating platforms (n = 13) using their preferred untargeted method. The results were analyzed in terms of the detectability of compounds, differences in the performance of various instruments, and the reliability of the identifications. Based on the results, we defined analytical criteria for a successful analysis of plant food bioactives. Furthermore, the results presented in the current study will serve as a basis for an optimized consensus method.

Title

Interoperable and scalable metabolomics data analysis with microservices

Authors

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Abstract text

Life scientists working with big data such as genomics, proteomics and metabolomics are beginning to experience numerous difficulties of handling, processing and moving information that has not been experienced before. These intensive computational tasks cannot be tackled by regular laptops and workstations. The high demand for more resources and increased flexibility has even turned the well-known shared high-performance computing unresponsive.

Cloud computing together with microservices architecture offer a compelling alternative to these old models, with the possibility to instantiate and configure on-demand resources such as virtual computers, networks, and storage, together with operating systems and isolated software tools.

We have developed an architecture which uses components for data analysis encapsulated as microservices connected into operating computational workflows. This solution provides users with complete, ready-to-run, reproducible and scalable data analysis environment that can be easily deployed on desktop computers as well as public and private clouds, without requiring special IT skills from the user.

We showcase the capability of this architecture using four demonstrators: in the first demonstrator we reproduce a large metabolomics data analysis and show how it can be effortlessly scaled up on cloud computing resources with little efficiency loss. In this case, 1092 computationally intensive tasks were performed in less than four hours which otherwise could have taken days to finish on a workstation. In the second demonstrator we provide new mass-spectrometry data from multiple sclerosis patients, and present for the first time, to the best of our knowledge, a complete start-to-end analysis of untargeted mass-spectrometry and identified novel biomarkers. The last two demonstrators describe NMR workflow and fluxomics workflows, emphasizing that microservice architecture is domain-agnostic.

The four demonstrators show the versatility, applicability and scalability of our method in metabolomics; however, the methodology can be applied to all scientific disciplines, paving the way towards for large-scale integrative science.

1st Nordic Metabolomics Conference
Örebro, August 26-28, 2018

Title
Targeted metabolomics applied to heart failure patients – a pilot study

Authors
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Abstract text

Targeted metabolomics was performed at AstraZeneca on a pilot study with plasma samples from heart failure (HF) patients in the 4D heart failure PREFERS project for improvement of heart failure management and research in Stockholm, initiated by the Karolinska Institute and Stockholm County. The patients were divided in three groups: HF with reduced ejection fraction (HFrEF, n=13), HF with preserved ejection fraction (HFpEF, n=22) and a HF control (n=41) group. All 76 patients had coronary artery bypass surgery performed.

The effective analysis of polar ionic metabolites by LC-MS, represents an analytical challenge for metabolic profiling. Ion-pair-chromatography (IPC) coupled to negative-electrospray-ionization tandem-mass spectrometry (1) was used to profile 100+ endogenous metabolic intermediates in human plasma. A complementary method based on hydrophilic interaction chromatography (HILIC) positive-electrospray-ionization tandem-mass spectrometry was developed covering another 70+ metabolites. By combining these two platforms metabolites representing amino acids, phosphorylated sugars, citric acid cycle intermediates, glycolytic intermediates, acyl carnitines, lysophospholipids, and nucleosides were covered.

There was no clear metabolite phenotype separation between normal, HFpEF and HFrEF patients based on targeted metabolomics but two metabolites were differentially abundant between HFrEF patients and controls and survived adjustment for multiple testing. There was also significant pair-wise separation between groups for more than 15 metabolites.

In conclusion, targeted metabolomics was successfully applied to the heart failure pilot study. Results were in-line with published data, confirming the capability of the platform to support future work on an expanded cohort aiming at identifying dysregulated metabolic pathways to advance the understanding of heart failure pathophysiology in search for new treatments and biomarkers.

(1) Michopoulos, F *et al*. Targeted profiling of polar intracellular metabolites using ion-pair-high performance liquid chromatography coupled to tandem mass spectrometry: applications to serum, urine and tissue extracts. J Chromatogr. A. 2014 Jul 4;1349:60-8.

Title
Accurate and Confident Metabolic Phenotyping
Combining a Standardized and Quantitative Targeted Assay with Orbitrap™ Technology

Authors (presenting author underlined)
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Abstract text

Multiplexed analysis by targeted metabolomics provides a quantitative snapshot of the metabolic phenotype. Targeted and standardized assays are used in research to improve biological insights, monitor disease biomarker signatures, and enable efforts in personalized medicine. A standardized, quantitative, rapid targeted metabolomics approach (**Absolute*IDQ*® p400 HR Kit**) covering the metabolites necessary for the assessment of the central metabolic pathways based on amino acids, acylcarnitines, hexoses, biogenic amines, and lipids in plasma, urine, and tissues was developed, combining LC-MS with FIA-MS analysis. Traditionally, quantitative analyses have been carried out on triple quadrupole MS instruments using MRM technology. The high resolution, accurate mass (HRAM) technology on OrbitrapTM instruments, on the other hand, has predominantly been used in profiling studies. Here, we investigate the coupling of a standardized, quantitative assay with the OrbitrapTM technology to demonstrate the power of multiplexed quantitative metabolome analysis, the impact of improved resolution, and increased metabolite coverage

Title

Metabolomics and Galaxy: Tools and workflows for increased reproducibility and transparency of metabolomics data processing and analysis in PhenoMeNal

Authors (presenting author underlined)
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Abstract text
Metabolomics data processing and analysis workflows are often complex and include many open source tools, each with their own dependencies, resource requirements and scripting languages. Configuring these tools is often complicated, especially for those untrained in informatics. Furthermore, many tools require users to input parameters that can significantly affect outputs and performance, though reporting of these parameters is not always clear. These challenges, faced when using metabolomics data processing workflows, hinder the reproducibility of metabolomics research, which must strive towards making data processing workflows more accessible and better reported.

The Galaxy Project [galaxyproject.org], originally developed for the genomics community, offers an open web-based platform enabling developers to make data processing tools and workflows available via an intuitive graphical user interface. Over the past few years this platform has been expanded, through projects including Workflow4Metabolomics [workflow4metabolomics.org], PhenoMeNal [phenomenal-h2020.eu], and MetaboFlow [metaboflow.org], to include many useful and powerful metabolomics tools. Tools included in this platform can be incorporated into defined and shareable workflows, helping to improve reproducibility and to extend their accessibility and utility by the wider scientific community.

Title
Once upon a time or the same story every day? Factors influencing blood-based metabolomics diagnosis of multiple sclerosis.

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Abstract text

When devising any diagnostic test, it is important to not only ensure accurate diagnosis in the presence of confounding factors but also to ensure the test is practical to carry out in a clinical setting. We have demonstrated, using nuclear magnetic resonance (NMR) spectroscopy on plasma/serum samples, that it is possible to distinguish between patients with multiple sclerosis (MS) and neuromyelitis optica (NMO). Using this same approach, we have also shown that we can separate patients with relapsing-remitting MS (RRMS) from secondary progressive MS (SPMS). As the metabolome is dynamic, variation in collection and processing of samples can affect the NMR metabolite profile. While the extent of the variation introduced because of freeze-thaw cycles, sample storage temperature, and time of last meal has been described previously, in general there is little known about how this variation can affect the accuracy of diagnostic models. To successfully translate our research findings into the clinics, we investigated if these factors have a significant impact on the diagnostic accuracy of our MS models. Plasma and serum samples were obtained from patients diagnosed with RRMS, SPMS and NMO, along with samples from healthy volunteers. Samples were analyzed using NMR spectroscopy and orthogonal partial-least squares discriminatory analysis (OPLS-DA) was used to identify significant differences in the metabolite concentrations and to produce predictive mathematical models that stratified between disease groups. We found that blood samples can be left up to 150 min before erythrocyte separation without introducing appreciable variation in the metabolite profile. In addition, NMR samples can be left at room temperature for up to 8 hours before the diagnostic accuracy of the test decreases. This observation was lipoprotein-dependent with VLDL converting to HDL when samples were left for longer periods of time at room temperature. While an additional freeze-thaw cycle was found to introduce more variation in the metabolite profile, this was less than interpersonal biological variation. Combined, these data suggest that our multivariate diagnostic models can perform accurately even when considerable variation in sample collection and processing are introduced. This has allowed us to define the tolerance limits of our diagnostic models and illustrates the huge translatable potential for clinical use.

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Title
A High Throughput Single Platform For High Throughput Quantitative MultiOmic Studies

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Abstract text

A high throughput targeted UPLC-MS/MS single platform, employing a reversed-phase gradient separation, has been developed for the quantification/monitoring of small molecule metabolites, lipids and peptides. The platform employs a single LC column and mobile phase combination which allows the analysis of multiple analyte classes with either positive or negative ion MRM detection.

The use of metabolic profiling (metabonomics/metabolomics) to discover biomarkers of organismal response to environmental and physiological change is now widespread. In biomedical applications metabolic profiling is being deployed as a method for finding novel, mechanistic, biomarkers of disease with obvious potential for improving diagnosis, and patient stratification. Hypothesis driven metabolomics delivers detailed qualitative and quantitative analysis on specific pathways or classes of metabolites, allowing researchers to analyse the effects of disease or treatments in greater detail.

These targeted assays usually employ "bespoke" methods which are optimized for each pathway or metabolic class making multiplexing assays difficult. We have developed a single analytical LC-MS/MS platform which is rapid, simple and reliable. The methodology employs a single LC column / mobile-phase combination which facilitate bile acids, biogenic amine, free fatty acids, acyl carnitines, lipids and 100 protein panel. This single platform approach has been employed for the analysis of plasma from a liver cancer study, showing excellent throughput and sensitivity.

Metabolomics data analysis and modelling approaches

Title
Multivariate risk modelling using 'triplots' for integrated analysis of exposures, metabolome and disease risk.

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Abstract text

In epidemiological research, associations between environmental exposures, such as self-reported diet or environmental pollutants, and outcomes are often investigated in nested case-control studies using conditional logistic regression (CLR). This is a mature technique, where calculated odds ratios can be adjusted for covariates, such as age, gender and body mass index. CLR is normally performed to estimate odds ratios per single exposures, but has also been performed based on combined exposures, including metabolites, from e.g. principal component analysis (PCA) or other reduced rank/component-based modelling techniques. However, wide-spread use of multivariate risk modelling is hindered by lack of effective tools for visualization and interpretation of findings.

We have therefore developed a novel tool for multivariate risk modelling and visualization that combines exposures, metabolome and disease risk in a 'triplot' approach. The core of this approach is a PCA of metabolites (or metabolomic features). The metabolite component scores are correlated with single or multiple exposures, such as self-reported diet or environmental pollutants, and also used as independent variables in CLR. A multivariate and directly interpretable visualization of associations of the metabolome with the exposures is then obtained by superimposing the metabolite loading plot with exposure correlations and CLR odds ratios.

We demonstrate the applicability of the 'triplot' approach for multivariate risk modelling, using data from two research projects aiming to investigate how the plasma metabolome and the risk of developing type 2 diabetes (T2D) are affected by exposures to either Healthy Nordic Diet or to perfluoroalkyl substances (PFAS; a class of persistent, bioaccumulative organic pollutants). These studies were performed in a nested case-control study design within the Swedish prospective VIP cohort. Using the 'triplot' approach we show (i) an association between dietary pattern and T2D risk, but that this association was not successfully captured by established diet scores for healthy Nordic eating and; (ii) that PFAS exposures were grouped into two clusters with distinctly different metabolomic fingerprints as well as different associations with T2D. The 'triplot' approach for multivariate risk modelling, was thus shown to aid in visualization and interpretation of multivariate risk modelling for combined exposures.

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ABSTRACT

Title
Metabolomics analysis and modelling shows sub-groups of trauma patient and suggests potential interventions

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Abstract text
Trauma is one of the leading causes of death worldwide (1). Endothelial damage has been shown to contribute to trauma outcome (2). This project has combined extensive measurements of metabolites with the use of an endothelial cell genome scale metabolic model to contextualise and interpret the data.
We have used liquid chromatography mass spectrometry (LC-MS) to analyse plasma samples from trauma patients on admission to Rigshopitalet Copenhagen. Metabolomic analysis of this data has shown that trauma patients are metabolically distinct from age and gender matched controls. Twenty-two metabolites have been identified as differentiating between patients and controls, these include amino acids; glutamine, leucine, and kynurenine, sugars; glucose, purines; adenosine, and fatty acids; linolenic acid. Further we have identified metabolically distinct sub-groups of patients. These sub-groups are largely distinguished by their fatty acid metabolism. These sub-groups also have some distinct clinical features, suggesting that they may be clinically relevant.
The endothelial genome scale metabolic model iEC2812 has previously been used to examine data from sepsis studies (3). This model has been updated to better account for the metabolism of functionally relevant glycocalyx components. The new trauma patient plasma metabolic measurements have been used to constrain the uptake and secretion fluxes of this model. Preliminary results suggest that differences in metabolism between the groups are in line with cell culture observations of damaged endothelium with regards to acetyl-CoA production, tryptophan metabolism and heparan-sulphate proteoglycan metabolism. Modelling has also suggested that alterations to carnitine, sphingolipid, fatty acid and glycan metabolism may be of benefit to the sickest trauma patients.
This approach combining metabolomics data with genome scale modelling has allowed us to contextualise a complex data set. It is hoped that this approach will lead to practical hypotheses for trauma interventions.

1. Mock C. Strengthening Care for the Injured Globally. J Trauma Inj Infect Crit Care. 2011 Jun;70(6):1307–16. PubMed PMID: 21817967
2. Pär Ingemar Johansson; Sisse R Ostrowksi;, Stensbakke J, Johansson P, Stensballe J, Ostrowski S. SHock INduced Endotheliopathy (SHINE) in acute critical illness - a unifying pathophysiologic mechanism. Crit Care. BioMed Central; 2017 Dec 9;21(25). PubMed PMID: 28179016
3. Mcgarrity S, Anuforo Ó, Halldórsson H, Bergmann A, Halldórsson S, Palsson S, et al. Metabolic systems analysis of LPS induced endothelial dysfunction applied to sepsis patient stratification. Sci Rep. 2018;In Press.

Molecular physiology in health and diseases

Title
Translational metabolomics approach to unravel nonalcoholic fatty liver disease diversity: learning from mouse models

Authors (presenting author underlined)
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Abstract text

Background and Aims:
Nonalcoholic steatohepatitis (NASH) is a histological definition that groups together defects in diverse biochemical processes causing hepatic fat accumulation, inflammation, necrosis and fibrosis. Increasing evidence points to different subtypes of nonalcoholic fatty liver disease (NAFLD) which progress to NASH and fibrosis at different rates and may respond differently to treatment. In addition, NASH therapy is being developed under the "one drug treats all patients" paradigm. The identification of the types of mechanisms leading to NASH and the discovery of non-invasive biomarkers of NASH subtypes are central for the development of effective treatments and precise diagnosis.
This study aims to capture the metabolic signature of different NASH subtypes through a translational research.

Materials & Methods:
We have analyzed the serum metabolome of three mouse models of NASH: Germline methionine adenosyltransferase 1a knockout (Mat1a$^{-/-}$), WT mice fed a methionine, choline deficient (MCD) diet, and high-fat fed Ldlr$^{-/-}$.Leiden mice. Then, murine serum metabolomic signatures were used as comparators to serum metabolome of a cohort of 535 individuals with biopsy proven diagnosis of NAFLD (353 with a diagnosis of simple steatosis and 182 with a diagnosis of NASH). For that, we performed a Silhouette cluster analysis and validated the process in 1,000-fold repetition of a random partition (50/50) of samples into two cohorts with equal proportional representation of steatosis/NASH. The frequency distribution of NAFLD patients into subtypes and of metabolites that significantly differentiated between NASH and steatosis per subtype was calculated.

Results:
The findings of the comparison between murine and human metabolome indicate the existence of two major human NAFLD phenotypes, each of them characterized by a specific metabolic alteration. The first phenotype comprised 40% of the patients, showing a metabolic profile compatible with low hepatic S-adenosylmethionine (SAMe), impaired very-low-density lipoproteins (VLDL) secretion, increased fatty acid uptake and normal de novo lipogenesis (DNL). The second phenotype included 32% of the patients, showing a metabolic profile compatible with normal SAMe and VLDL secretion, and increased DNL. Treatment with obeticholic acid improved NASH in the high-fat fed Ldlr$^{-/-}$.Leiden mouse model but had no effect in the MCD model, confirming the need of developing a personalized treatment in NASH.

Conclusions:
These results indicate that the traditional, mainly pathology-driven classification of NAFLD/NASH, can be refined and perhaps represented by a metabolomic-based classification. In addition, serum metabolomic profiling will give the opportunity to identify those patients that respond better to a specific treatment.

Title

Determination of the circadian metabolome in hemolymph of Drosophila melanogaster

Authors

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Abstract text

In the natural environment, every organism encounters periodic light-dark cycles. Therefore, proper timing using internal clocks is beneficial to adjust physiology and behaviour to approximately 24 hours. Circadian behaviour of *Drosophila melanogaster* has been broadly studied. For example wild-type (Canton-S) is active in the morning and evening and makes a siesta at noon. However, the function of the clock network components in daily timing of metabolism in the fruit fly is poorly understood. As a result, I aim to discover metabolic pathways or metabolites that oscillate diurnally in the insect hemolymph (analogous to blood). To achieve this, wild type flies kept at 12-12 hour light/dark cycle and fed sucrose *ad libitum* were sampled every three hours. The lipidome of the hemolymph was analyzed with ultra-performance liquid chromatography coupled to time-of-flight mass spectrometry (UPLC-MS). Experiments consistently revealed that levels of diacylglycerol (DAG) seem to oscillate in the hemolymph of 5 days old male flies of wild-type (Canton-S). Furthermore, the feeding behavior of these flies on sucrose under 12-12 hour light/dark cycle showed a rhythm which may play a role in DAG oscillation.

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ABSTRACT

Title
Ad libitum feeding alters several metabolic pathways in Yucatan minipigs - multi-compartmental metabolomics in animal obesity models

Authors (presenting author underlined)
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Abstract text

Miniature pig models for human metabolic disorders such as obesity and metabolic syndrome are gaining popularity. In order to address current gaps in knowledge regarding the use of miniature swine as models for obesity and other metabolic disorders of human interest, we used non-targeted LC-MS metabolomics to investigate the effects of energy dense diets in development of obesity in female Yucatan minipigs. We compared the use of an ad libitum feeding strategy (n=8) to a restrictive approach (n=9) to observe the effects of an accelerated feed intake on the phenotype and metabolome. Serum, urine and liver tissues were collected at slaughter after five months of dietary intervention. Samples were prepared for non-targeted metabolomics and analyzed using a Dionex UltiMate 3000 UPLC coupled with an Impact HD Quadrupole Time-of-Flight (QTOF) mass spectrometer. Chromatographic separation of compounds was performed using an Acquity HSS T3 Waters column. Overall, ad libitum feeding altered the metabolic profile of these three major compartments (liver, urine, serum). Our study revealed changes at the hepatic level, where an increased activity in the glutathione pathway (glutathione, P=0.001; L-glutamate, P=0.04) could indicate a protective response to the high intake of energy dense feed. This has previously been reported only in alcohol-induced steatohepatitis in swine. Several metabolites were identified in both serum and urine indicating common biological feedback and changes in metabolic pathways affected by development of obesity: pantothenate and CoA biosynthesis and tryptophan metabolism. Pantothenic acid (serum, P<0.01; urine, P=0.001) was increased with ad libitum feeding, a possible physiological response to the lipogenic effect of the western-style diet. Tryptophan (TRP, P<0.01) and indolelactic acid (P=0.03) were upregulated in the serum of ad libitum fed animals compared to restrictive fed animals and urine revealed other degradation products of TRP: N-acetyl-tryptophan (P<0.01), kynurenic acid (P<0.01), xanthurenic acid (P<0.01), 3-methyldioxyindole (P=0.06). As TRP is the main precursor of serotonin synthesis, it is worth considering the dietary intake of TRP and its effects on serotonin-appetite control in relation to ad libitum feeding. In this multi compartmental approach, we were able to identify several metabolites and their respective pathways that were changed across different tissues. Many of these metabolites have been correlated in previous studies with the progression of obesity and indicative of metabolic syndrome, confirming the usefulness of non-targeted metabolomics as an explorative tool in dietary intervention studies.

Circulating endocannabinoids are dysregulated with respect to central CB1-receptor availability in male patients with first episode psychosis.

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There is an established link between psychosis and metabolic abnormalities such as altered glucose metabolism and dyslipidemia. However, the mechanism by which these metabolic changes occur remains unclear. Although antipsychotic drugs can contribute to the metabolic changes there is evidence that the alterations precede antipsychotic treatment. Metabolomics approaches have been used to quantify the metabolic changes occurring in first episode psychosis. Identifying dysregulated metabolism, could be used to predict the patients at risk of developing metabolic co-morbidities.

Using a quantitative liquid-chromatography triple-quadrupole mass spectrometry assay, nine endogenous endocannabinoids or related structures were measured from serum obtained from first episode psychosis patients (FEP, n=8) and healthy controls (HC, n=10). After serum sampling brain CB1R availability was quantified in the same individuals using positron emission tomography (PET) and specific cannabinoid-1 receptor (CB1R) tracer [18F]FMPEP-d2.

Circulating levels of arachidonic acid (p=0.02) and oleyl ethanolamide (p=0.04) were reduced in the FEP individuals. In order to compare the levels of circulating endocannabinoids to the brain CB1R availability PLS regression modelling was used. In HC there was strong association of arachindonoyl glycerol (1+2), stearoyl ethanolamide and palmitoyl ethanolamide with the CB1R availability in the grey matter of the hippocampus (R^2_{CV}=0.51) which was lost in the FEP patients (R^2_{CV}=0.10).

The dysregulation of circulating endocannabinoids in the circulation compared to CB1R following a FEP highlights a possible mechanism by which metabolic co-morbidities occur in psychosis. Despite the small number of patients in this study there is a clear dysregulation of the endocannabinoid system in patients with FEP.

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Title
Serum metabolites associate with CT findings following TBI

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Abstract text

There is a need to rapidly detect patients with traumatic brain injury (TBI) who require head computed tomography (CT). Given the energy crisis in the brain following TBI, we hypothesized that serum metabolomics would be a useful tool for developing a set of biomarkers to determine the need for CT and to distinguish between different types of injuries observed.
Logistic regression models using metabolite data from the discovery cohort (n=144, Turku, Finland) were used to distinguish between patients with traumatic intracranial findings and negative findings on head CT. The resultant models were then tested in the validation cohort (n=66, Cambridge, UK). The levels of glial fibrillary acidic protein and ubiquitin C-terminal hydrolase-L1 were also quantified in the serum from the same patients.
Despite there being significant differences in the protein biomarkers in patients with TBI, the model that determined the need for a CT scan validated poorly (AUC=0.64: Cambridge patients). However, using a combination of six metabolites (two amino acids, three sugar derivatives and one ketoacid) it was possible to discriminate patients with intracranial abnormalities on CT and patients with a normal CT (AUC=0.77 in Turku patients and AUC=0.73 in Cambridge patients). Furthermore, a combination of three metabolites could distinguish between diffuse brain injuries and mass lesions (AUC=0.87 in Turku patients and AUC=0.68 in Cambridge patients).
This study identifies a set of validated serum polar metabolites, which associate with the need for a CT scan. Additionally, serum metabolites can also predict the nature of the brain injury. These metabolite markers may prevent unnecessary CT scans, thus reducing the cost of diagnostics and radiation load.

A metabolomics approach to uncover the role of sphingolipids in metabolic diseases

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Besides being precursors in sphingolipid biosynthesis, ceramides are increasingly being recognized as universal regulators of biological stress responses, and thus been associated with the development of several pathologies. Ceramides accumulate in obese and insulin resistant individuals. Once ceramide levels rise above a critical threshold, tissues become insulin resistant and thus compromise nutrient uptake and storage. Prevention of ceramide accumulation in rodents improves insulin sensitivity and prevents the onset of metabolic disorders including diabetes, steatohepatitis and atherosclerosis. However, despite of the importance of sphingolipids in cellular homeostasis, the molecular mechanisms of how ceramides and other sphingolipids affect intracellular metabolism still remain largely unsolved. Here we have used mass spectrometry-based metabolomics and cellular and animal models to untangle how sphingolipids regulate intracellular metabolism. Mouse C2C12 myoblasts, AML-12 hepatocytes and 3T3-L1 adipocytes were cultured in the absence or presence of palmitate (a precursor of the *de novo* sphingolipid pathway) and myriocin, an inhibitor of serine palmitoyl transferase-1, the rate-limiting enzyme of *de novo* ceramide synthesis. By LC-MS we identified several lipid species and metabolites, and found that palmitic acid has wide effects on the abundance of intracellular metabolites. Inhibition of the *de novo* ceramide synthesis by myriocin reverted the changes of certain metabolites (e.g. TCA cycle and purine metabolism intermediates). We also find that myriocin counteracts diet-induced obesity and insulin resistance when supplemented to mice. Our LC-MS analyses show how myriocin affects metabolism in normal and in diet-induced obese mice, and thus provide novel insights into how sphingolipids regulate metabolism.

Title

Fit B: personalized metabolic responses to physical activity for improved fitness and training

Authors (presenting author <u>underlined</u>)

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Abstract text

Increasing physical activity (PA) is currently widespread among the population. Thus, in addition to the professional, the amateur and the physically inactive persons being engaged for many different reasons, PA is considered a major driver for optimal health achievement throughout life, with special interest in our ageing society.

It is widely known and scientifically accepted that two of the most influential factors for full achievement of PA benefits are a proper diet and an adequate training. All athletes tend to follow specific diets and training programs according to the type of sport performed, but still, not everyone responds the same way to the same diet or the same training. To solve these pitfalls, amplifying the health benefits of PA and approaching it to the general consumer, one have to take into account the enormous diversity of metabolic responses towards PA. Physiologically, PA and exercise are considered stressors. Fit B is a test based on metabolic biomarkers for giving personalized nutrition and training tools towards PA.

The test uses two small blood samples from a finger puncture which are collected in a special device by the athlete himself just before and after the PA. The samples can then be delivered to our facilities where they are analyzed in a LC-MS system. Both the device and the compounds used as biomarkers have been selected due to its stability during the transport, storage and analysis to ensure reliability. The athlete also receives access to an own online platform where medical history, data about lifestyle and the PA of interest is recorded and analyzed to give a more accurate and personalized recommendations.

Preliminary data shows that these biomarkers could be associated with parameters related both to the PA and the athlete, like the intensity of the exercise, the athlete previous training or a component of fatigue. This could allow a monitoring of the athlete's response to training or diet.

Biomeb is a biotechnology company, a spin-off of the University of Lleida, whose mission is to provide personalized medicine, providing new products and services to society that mean a breakthrough in the prevention, diagnosis and personalized treatment of different pathologies. Our company is founded by PhD from diverse backgrounds in physiology and biochemistry, and our collaborators are experts in healthcare, statistics, bioinformatics, information technologies and software development.

Title

Novel *Borrelia* infection biomarker identification by nuclear magnetic resonance spectroscopy

Authors (presenting author underlined)

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Abstract text

Lyme borreliosis, is the most common tick-borne infection in the northern hemisphere (1). Borrelia diagnostics is currently based on clinical evaluation of the disease and laboratory testing. Local infection cases are diagnosed by clinical evaluation, but in disseminated cases laboratory testing is required. Serology is the main method of laboratory testing and is performed by ELISA and immunoblot methods. Current serology detects only 20-50% of dissemintade infections. Problematic is also detecting reinfections because of antibody levels which stay high after infection. (2)

In this study novel Borrelia infection biomarkers were searched for by NMR method using serum and urine of infected mice as sample material. Differences in small molecular weight compounds between control and infected samples were the interest of this study.

The study was performed in two separate experiments. In the first experiment 16 female C3H/HENhsd mice, and in the second experiments 40 mice were utilized (half infected half control in both studies). Mice were infected with Borrelia bacteria and were followed up for 4 weeks. At the end of the experiments, total blood and urine samples were collected. After collection of the samples, urine samples of the same mouse were pooled together. Blood samples were centrifuged and serum was collected. All samples were stored at -80 °C.

NMR analysis of the samples was performed by Bruker Avance III 600 instrumen. Measurements of the serum samples were conducted by CPMG pulse sequence and urine samples by 1D-NOESY pulse sequence. NMR data handling was done by Bruker TopSpin 3.5 pl3 software, Bruker Amix 3.9.15 software and Umetrics Simca-P+ 12.0.1 software. Peak alignment of the signals was made by matlab base icoshift algorithm.

Small molecule metabolite differences between infected and control mice were observed in both serum and urine samples. Major differences in the concentrations of certain amino acids, immune system energy metabolites and purine synthesis metabolites bath in urine and serum.

This study clearly shows that there are metabolic differences between Borrelia infected and control. These differences can mostly be characterized as common infection differences, and the Borrelia specificity of the differences must be confirmed in the further studies. The most interesting findings are differences in the concentrations of amino acids, especially in concentration of phenylalanine.

1.   Stanek G, Wormser GP, Gray J, Strle F. 2012. Lyme borreliosis. Lancet 379:461-73.
2.   Aguero-Rosenfeld ME, Wang G, Schwartz I, Wormser GP. 2005. Diagnosis of lyme borreliosis. Clin Microbiol Rev 18:484-509.

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ABSTRACT

LC-MS analysis of endogenous and ^{13}C-labelled TCA-centered metabolites in cerebral microdialysis

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Abstract

Cerebral microdialysis allows continuous sampling of brain metabolites by using a dialysis catheter inserted into the interstitium of the brain. By perfusing with Ringer's solution through a catheter, small molecules are exchanged between the brain interstitium and the perfusate. Cerebral microdialysis of glucose, lactate and pyruvate is used to monitor patients with compromised blood flow. Transient ischemic events, a result of compromised blood flow, cause a drop in glucose and rapid rise in lactate. Following such events the lactate/pyruvate ratio may remain elevated for prolonged time, which has been interpreted as a metabolic marker of mitochondrial dysfunction. Here we propose a novel microdialysis LC-MS approach where metabolites essential for the tricarboxylic acid cycle are monitored along with incorporation of ^{13}C upon continuous perfusion with ^{13}C-labelled succinate. The approach was validated using two different rat models: a) Mitochondrial inhibition induced by malonate, an inhibitor of succinate dehydrogenase and b) transient cerebral ischemia induced by local application of endothelin-1.

In the malonate model, TCA metabolites could be detected in the perfusate and the expected changes upon inhibition of succinate dehydrogenase were observed (i.e. increase of endogenous succinate and decrease of fumaric acid and malic acid). The inhibition was further elaborated by incorporation of ^{13}C into specific compounds. In the endothelin-1 model compromised blood flow was shown by a transient decrease in glucose and increase in lactate. Furthermore, hypoperfusion-induced alterations in TCA metabolites showed opposite effects of non-labelled and ^{13}C-labelled, illustrating immediate effects of endothelin-induced vasoconstriction on release of intracellular compounds versus *de novo* synthesis (decrease of ^{13}C-labelled metabolites).

[13]C-labelled microdialysis using perfusion with ^{13}C-succinate is a promising tool to monitor mitochondrial dysfunction *in vivo,* as more metabolites can be detected due to the systems high sensitivity. As illustrated by the transient ischemia model, the analysis of 13C incorporation provides a further layer of information important for identifying alterations in metabolites from e.g. neurointensive care patients.

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Title
Integration of magnetic resonance imaging, and protein and metabolite measurements to enable early diagnosis of secondary progressive multiple sclerosis

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Abstract text

Multiple sclerosis (MS) is a debilitating neurological disease affecting approximately 2.5 million individuals' world-wide. The course of MS is heterogeneous and involves an early, predominantly inflammatory disease phase of relapsing-remitting MS (RRMS). After a variable time, RRMS evolves into a progressively degenerative phenotype (secondary progressive MS, SPMS) with neurodegeneration, brain atrophy and accumulation of disability. At present, SPMS is diagnosed retrospectively and unmet needs are methods for early detection and monitoring of SPMS.

The aim of this study was to investigate if a combination of different types of data could be used to improve on diagnosis of SPMS and predict disease progression.

We integrated clinical and magnetic resonance imaging (MRI) data with data from protein and metabolite measurements of cerebrospinal fluid (CSF) and developed a method to sift through all the variables to establish a small set of highly informative measurements. This prospective study included 16 SPMS patients, 30 RRMS patients and 10 controls. Protein concentrations were quantitated with multiplexed fluorescent bead-based immunoassays and ELISA. The metabolome was recorded using liquid chromatography-mass spectrometry. Patients were followed for a mean of 5 ± 1.4 years.

By combining eleven different variables (three MRI, six protein and two metabolite), SPMS patients could be identified with high confidence, superior to any of the single measurements alone. Two proteins and two metabolites were associated with the disease progression. Four RRMS patients converted to SPMS during the follow-up period. Three of them clearly displayed phenotypes similar to SPMS, two-to-three years prior to the SPMS diagnosis, whereas the remaining one displayed a RRMS phenotypes.

Our results suggest that a combinatorial approach may aid in early identification of patients at risk for SPMS and rapid progression.

Title

Diet or surgery - A multi-platform metabolomics approach to investigate metabolic changes induced by caloric restriction and gastric bypass surgery

Authors (presenting author underlined)

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Abstract text

Diabetes is a devastating, chronic metabolic disease with vastly increasing numbers of incidences. Type 2 diabetes (T2D) develops when the pancreatic beta-cells fail to compensate for reduced insulin sensitivity. Roux-en-Y gastric bypass surgery (RYGB) is an effective approach to induce weight loss and T2D remission in obese individuals. The mechanisms driving T2D remission are, however, not fully understood. Recent studies suggest that a very low calorie diet results in similar T2D remission rates as RYGB. Therefore, it is debated whether caloric restriction may induce the effects observed after RYGB.

In this study, we used blood plasma from 19 morbidly obese individuals with (n=10) or without T2D (n=9) to investigate the alterations in metabolite levels after caloric restriction and after a subsequent RYGB. We applied three complementary metabolomic methods based on ultrahigh performance liquid chromatography (UHPLC) coupled to quadrupole time-of-flight mass spectrometric (QTOF-MS). Together, these methods cover >300 identified metabolites, including a variety of lipids, low molecular weight polar metabolites, such as amino acids, and metabolites with intermediate polarity, such as acylcarnitines. Raw data were processed and peaks identified using a developed pipeline, combining commercially and open-source analysis tools, and both open-source and in-house libraries. We applied multivariate data analyses techniques such as orthogonal projections to latent structures discriminant analysis and mixed linear regression models to analyse the data.

Our data suggest that the effects of dietary caloric restriction, and the combination of diet and RYGB, elicit similar changes in the fasting metabolic state. A number of analysed metabolites, however, were altered after RYGB only, suggesting different metabolic changes than induced by caloric restriction. Using this approach, we aim to identify specific metabolites that will aid to unravel the mechanisms driving T2D remission

Title
The effect of faecal microbiota transfer on the bacterial metabolite profile in irritable bowel syndrome patients

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Abstract text
Faecal microbiota transfer (FMT) is used as a method to try to exchange a disturbed faecal microbiota with a healthy one. Irritable bowel syndrome (IBS) is a disorder in which a disturbed faecal microbiota might play a role in the pathophysiology, and therefore FMT has been suggested as potential treatment. The aim of this study was to investigate the effect of FMT on selected faecal bacterial metabolites in IBS patients over time, their correlation patterns, and to assess if these metabolites correlate with symptom scores.

In a previously conducted placebo-controlled clinical trial, 16 IBS patients were treated with FMT, faecal samples were collected 1 day before FMT, and 3 days, 7 days, 2 weeks, 4 weeks, 8 weeks and 6 months after FMT. FMT consisted of either allogenic faecal material (stool from healthy donor) or autologous faecal material (own stool) administered in the coecum by colonoscopy. Acetic acid, propionic acid, isobutyric acid, butyric acid, 2-methylbutyric acid, isovaleric acid, valeric acid, methione, tyrosine, valine, leucine and isoleucine were measured by GC-MS spectrometry and corrected for faecal dry weight. IBS symptom scales GSRS-IBS and IBS-SSS were completed by the patients at the same time points as faecal samples were collected. Pearson correlations were calculated between group-wise averaged baseline-corrected metabolites, symptom scale data and time points. Differential network analysis was performed to compare correlations between the allogenic and autologous FMT group.

The differential network analysis showed that the short-chain fatty acids (SCFAs) butyric acid, isobutyric acid, 2-methylbutyric acid and propionic acid were positively correlated with time in the allogenic FMT group, which was not observed in the autologous FMT group. This indicates that IBS patients receiving an allogenic FMT had increasing levels of these SCFAs in their faecal samples over time compared to patients that received autologous FMT. Additionally, leucine, isoleucine, methione, valine, tryosine and acetic acid were positively correlated with GSRS-IBS score in the allogenic FMT group, other than in the autologous FMT group. This suggests that in the allogenic FMT group, higher GSRS-IBS scores were associated with higher levels of these metabolites compared to the autologous FMT group.

In this study we found that IBS patients receiving allogenic FMT had different interactions between SCFA and time points, and amino acids and symptom scores, compared to IBS patients receiving autologous FMT. Further research needs to be done to assess whether these changes in metabolites can be correlated to the allogenic faecal microbiota.

Differences in lipoprotein subclasses among elderly subjects with familial hypercholesterolemia with presence of coronary heart disease and among genders

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Coronary heart (CHD) caused by atherosclerosis is the main cause of mortality in Norway. Mild hypercholesterolemia is primarily lifestyle-induced, but more severe hypercholesterolemia is often caused by a genetic disposition such as familial hypercholesterolemia (FH). Subjects with FH are exposed to substantially elevated cholesterol levels from birth. More than 90% of the patients experience CVD during life, mean age of first myocardial infarction is 44 years, and mean age of cardiovascular death is 61 years. Effective cholesterol lowering treatment (statins) ideally from the age of 8-10 years is believed to reduce the risk of early morbidity and mortality. Statins, however, has only been wide-spread available the last 25 years. FH subjects who today are above 65 years did not receive statins until 45 years of age, and have thus "survived" much longer than the average FH patient even though exposed to elevated cholesterol levels for at least 45 years. These older FH subjects may therefore serve as a model to characterize markers of CVD resistance under hypercholesterolemic conditions. We aimed to investigate the effect of life-long exposure to elevated serum cholesterol in old subjects resistant and not resistant to the development CVD. We aimed to perform a lipid profile by doing a comprehensive metabolomics profiling using NMR technology in FH subjects older than 65 years.

Eighty-three subjects with FH were included in the study. Mean age was 70 years (min-max; 65-80 years), 48% were female and 39% had previously had coronary heart disease (CHD). Subjects with CHD had lower on-treatment total-, LDL- and HDL cholesterol than subjects without CHD due to more intense treatment. Analysis of lipoprotein subclasses showed that the concentration of extra-large and large HDL particles as well as HDL diameter, and cholesterol in extra-large and large HDL were significantly lower among the CHD-group, whereas IDL particle concentration were significantly higher. Women had significant higher on-treatment total-, LDL- and HDL cholesterol than males. IDL, large and medium LDL, extra-large, large and medium HDL as well as HDL diameter, and total cholesterol in HDL, HDL2 and HDL3 were significant higher in females than males. Women also had lower levels of the branched chain amino acids; isoleucine, leucine and valine than males. In conclusion, FH subjects without CHD were characterized with a more favourably lipid profile whereas women with FH were characterized by a more atherogenic profile.

Title
Metabolic profile alterations caused by heart specific PGC-1α deletion in mice

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Abstract text

Changes in the metabolism of several organs, including heart, liver and skeletal muscle, as well as circulating biomarkers have been found to be associated with heart failure in humans and research models. However, it is still unclear which of these changes originates from cardiomyocyte metabolic remodeling and which are induced secondarily by systemic factors. To investigate these questions we induced cardiac restricted metabolic changes by generating a cardiac-specific peroxisome proliferator-activated receptor- γ (PPAR-γ) coactivator-1α (PGC-1α) knockout mice (KO) to mimic metabolic dysregulation seen in heart failure. PGC-1α is a central regulator of heart energy metabolism and reduced PGC-1α expression has been linked to the metabolic alterations associated with heart failure (1). We characterized the PGC-1α KO-associated metabolite changes in the heart, circulation and peripheral tissues (liver and skeletal muscle) by performing a non-targeted LC-MS metabolite profiling analysis. Heart specific PGC-1α KO caused a progressive cardiomyopathy with cardiac dilatation leading to heart failure. Furthermore, we observed suppressed mitochondrial oxidation and reduced anaerobic metabolism in the isolated cardiomyocytes from the KO animals, as measured with Seahorse flux analyzer. KO hearts displayed a distinctive metabolite profile including metabolite changes in energy and phospholipid metabolism. For example, we observed depressed acetylation of both coenzyme A (CoA) and L-carnitine, as well as decreased levels of NAD+ demonstrating the reduced capacity of KO hearts to produce energy, in line with the Seahorse analysis. Some of the metabolite changes correlated strongly with the specific parameters of cardiac function measured by echocardiography. For example, changes in mitochondrial metabolites correlated with indirect and direct measures of cardiac contractile function including systolic left ventricle wall thickness, ejection fraction and cardiac output. Importantly, we did not observe any significant alterations in the metabolomes of the other measured tissues or in plasma. In conclusion, metabolic, functional and structural abnormalities associated with dilated cardiomyopathy were induced by the heart specific PGC-1α KO. The metabolic changes were limited to the cardiac tissue indicating that cardiomyocyte metabolic remodeling is not sufficient to evoke the body wide metabolic alterations usually associated with heart failure.

(1) Tuomainen and Tavi. The role of cardiac energy metabolism in cardiac hypertrophy and failure. Exp Cell Res. 2017 Nov 1;360(1):12-18.

Title
Aberrant T Cell Metabolism in Type 1 Diabetes

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Abstract text

Type 1 diabetes (T1D) is a chronic and potentially debilitating autoimmune disease characterized by T lymphocyte mediated destruction of the insulin-producing β cells of the pancreatic islets, insulinitis and lifelong dependence on injections of insulin. T1D is the prominent form of diabetes affecting children and adolescents and currently without an effective cure. One of the greatest challenges in developing therapies against T1D is the lack of understanding of the early disease etiology i.e. the events which precede the activation of the immune system against the β cells. We hypothesize that environmental factors, particularly metabolic, play a major role in driving the development of the autoimmune responses. This is supported by the fact that abnormalities in *e.g.* the levels certain circulating amino acids, Krebs cycle intermediates and lipid species between healthy control subjects and those progressing towards developing overt T1D, have been reported in several studies. It is also well documented that activation of naïve CD4+ T cells results in gross metabolic reprogramming of the proliferating cells while significant differences remain to persist between the T effector (Teff) cell subsets which drive immune responses and the immunosuppressive T regulatory (Treg) cells. We've thus performed a thorough metabolic characterization of the early steps of human T cell activation and differentiation using metabolomics, lipidomics and in silico metabolic modelling approaches. Based on an in-depth data mining of this vast dataset, we've identified a number prominent metabolic differences between the Teff and Treg cell subsets and designed strategies to affect the T cell differentiation pathways via modulating cell metabolism. Based on this evaluation and a priori knowledge of the metabolic abnormalities associated with T1D, we are testing a panel of metabolic interventions and their effects on the proliferation and polarization of naïve CD4+ cells into the mature T cell subsets. The ultimate aim of the project is the development of novel forms of dietary and drug mediated therapies against and for the prevention of T1D.

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Abstract

Investigation of missing values for untargeted LC-MS nutritional metabolomics studies: Comparison Analysis of novel Imputation methods

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Background: Metabolomics studies involve investigation of small molecules or metabolites, which are present in biological samples. The primary motivations behind this new '-omics' technology is to study alterations in metabolism under different conditions and profile as many metabolites as possible in the samples (metabolomes). Metabolomics is commonly applied in nutrition field to enhance the understanding of the effects of specific diets or food items on metabolic pathways and to seek potential biomarkers for food consumption. One of the most commonly used technology in metabolomics is liquid chromatography combined to mass spectrometry (LC-MS) which generates a vast amount of metabolomics data in short time. One of the challenges of LC-MS technologies are the missing values which can occur either because the concentration of some metabolites are below the detection limit of the instrument in use or because that particular metabolites are truly absent from the sample due to the effect from a specific diet. [1]. Furthermore, many of the classical statistical methods used to analyse these data require a complete data matrix. One solution to this is to use imputation methods that complete the dataset matrix by replacing missing values with plausible estimates, and therefore enable statistical analysis. Three types of missingness exists; MCAR, MAR and MNAR [2] and for each type of missingness numerous imputation methods [3] have been designed to estimate them. On the other hand, imputation methods have a major effect on the outcome of data analysis so a wrong choice can lead to biased results. Therefore, there is a need for novel imputation strategies that account for the type of missingness for each metabolic compound and selects the best imputation method for each of them.

Methods: We compare novel imputation methods that estimate the missing values in simulated and real nutritional metabolomics data. The comparison of the methods was done using Normalized mean squared error(NRMSE) .

Results: Based on our preliminary results, correctly detecting the type and amount of missingness and using this information to select a correct imputation method, has a great effect on the accuracy of the data imputation.

References

[1] Pedreschi, R., M. L. Hertog, S. C. Carpentier, J. Lammertyn, J. Robben, J. P. Noben, B. Panis, R. Swennen and B. M. Nicolai (2008). "Treatment of missing values for multivariate statistical analysis of gel-based proteomics data." Proteomics 8(7): 1371-1383.

[2] Rubin, D. B. (1976). "Inference and missing data." Biometrika 63(3): 581-592.

[3] García-Laencina, P., J.-L. Sancho-Gómez and A. Figueiras-Vidal (2010). "Pattern classification with missing data: a review." Neural Computing & Applications 19(2): 263-282.

Title
The circulating metabolome in the progression to islet autoimmunity and type 1 diabetes

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Abstract text

Type 1 diabetes (T1D) is a chronic autoimmune disease caused by specific destruction of the insulin-producing beta cell. Clinically pre-diabetic period in T1D is characterized by presence of beta cell specific autoantibodies. Intriguingly, earlier metabolomics study suggests specific metabolic disturbances before individuals precede to auto-immunity [1-3].

Here we combine lipidomics [4] and metabolomics approach to analyze circulating metabolites in a prospective series of plasma samples from 40 children who progressed to T1D (PT1D), 40 children who developed at least a single islet autoantibody but did not progress to T1D during the follow-up (P1Ab) and 40 matched controls (CTR). We found sphingomyelins and methionine to be persistently dysregulated in PT1D when compared to the P1Ab and CTR groups. Additionally, phosphatidylcholines and triacylglycerols were downregulated in PT1D as compared to P1Ab, while amino acids including glutamine, aspartic acid were downregulated in PT1D as compared to CTR at the age of 3 months. Furthermore, we found hydroxyphenyllactic acid, indole acetic acid, and 11-eicosenoic acid, metabolites of potential microbial origin, to be significantly downregulated at early age (3 and 6 months) preceding clinical T1D.

Our study support findings from earlier studies and suggests novel metabolic signatures that specifically characterize children who progressed to islet autoimmunity or overt T1D, respectively, which may be helpful in the identification of at-risk children before the initiation of autoimmunity.

References

1.   Oresic, M., et al., *Dysregulation of lipid and amino acid metabolism precedes islet autoimmunity in children who later progress to type 1 diabetes.* J. Exp. Med., 2008. **205**(13): p. 2975-84.
2.   La Torre, D., et al., *Decreased cord-blood phospholipids in young age–at–onset type 1 Diabetes.* Diabetes, 2013. **62**(11): p. 3951-3956.
3.   Orešič, M., et al., *Cord Serum Lipidome in Prediction of Islet Autoimmunity and Type 1 Diabetes.* Diabetes, 2013. **62**(9): p. 3268-3274.
4.   Lamichhane, S., et al., *Dynamics of Plasma Lipidome in Progression to Islet Autoimmunity and Type 1 Diabetes: Type 1 Diabetes Prediction and Prevention Study (DIPP).* bioRxiv, 2018 doi: 10.1101/294033

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Title
Evaluation of metabolic alterations in plasma from prostate cancer patients associated with disease aggressiveness.

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Abstract text

Prostate cancer is the most common cancer malignancy among Swedish men, with approximately 10 000 new cases diagnosed each year. It is imperative to detect the malignant cancer at an early stage in order to allow for a curable treatment. When the prostate cancer has spread outside the prostate and metastasized only palliative care is care given in order to improve the quality of life of patients. The discovery of new biomarkers for prostate cancer for detection of clinically significant tumors at a time-point when cure is possible are therefore needed.

The aim of this study is to identify metabolites in plasma associated with disease progression of prostate cancer. A further aim is to investigate metabolic differences before and after treatment with radical prostatectomy (surgical removal of the prostate), in association with biochemical relapse. Two sample cohorts are used in order to meet these two aims.

Our first study population consists of 63 cases of prostate cancer and 56 controls. Blood samples were drawn, from non-fasting subjects. Information on death and cause thereof, risk status etc where obtained with a follow up time of more than 12 years. Our second study population consists of paired blood samples drawn before and after radical prostatectomy respectively. The cohort consists of 62 prostate cancer patients that underwent surgery. The paired blood samples were drawn immediately before surgery and approximately three months after surgery. All blood samples were analyzed with GC/MS and LC/MS.

In order to identify the different metabolites associated with disease progression of prostate cancer, OPLS-DA were used. The most interesting metabolites or metabolite patterns are thereafter investigated further. Preliminary findings show a separation between controls and metastatic prostate cancer.

In the second study population the novel method OPLS-EP(1), a method designed to allow for comparison of paired samples, will be used, in order to evaluate the effect of the radical prostatectomy and relate this to the future outcome.

(1) Jonsson P, et al. Constrained randomization and multivariate effect projections improve information extraction and biomarker pattern discovery in metabolomics studies involving dependent samples. Metabolomics. 2015 Dec; 11(6):1667-78.

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ABSTRACT

Title
Lipidomic signatures of non-alcoholic fatty liver disease

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Abstract text

Nonalcoholic fatty liver disease (NAFLD) is a major risk factor leading to chronic liver disease and type 2 diabetes. Non-invasive diagnostic techniques for the different stages of NAFLD, such as steatosis, nonalcoholic steatohepatitis (NASH) and fibrosis, are currently unavailable and thus are an unmet medical need. In our previous studies, we successfully identified specific serum molecular lipid signatures which associate with the amount of liver fat (1) as well as with NASH (2).

Here, we take this further as we investigated serum lipidomic profiles in a clinical cohort of individuals (n = 688) in the European project Elucidating Pathways of Steatohepatitis (EPoS). The EPoS cohort comprised individuals at various stages of NAFLD (n = 666), including NASH (n = 661) and fibrosis (n = 511). In line with previous studies (1), we found that steatosis grade was strongly associated with the increase of certain triglycerides with low carbon number and double bond content as well as a decrease of specific phospholipids. As NAFLD progresses from an earlier steatosis state to a later, more severe fibrotic stage, fibrosis grades are also used as a clinical measure for assessing progression to and severity of NASH. Preliminary analysis of 511 of the cohort with graded presence of fibrosis versus those without, revealed that distinct relationships also exist between circulating lipids and fibrosis stage, the profile changing appreciably between steatosis and fibrosis.

In summary, our findings suggest that dysregulation of lipid metabolism in progressive stages of NAFLD is reflected in circulation and may thus hold diagnostic value as well as offer new insights about the NAFLD pathogenesis. Further analysis of these markers alone and in combination is warranted and is currently being undertaken to take these preliminary findings further with a view to assessing diagnostic utility of such markers.

(1) Orešič M, et al. Prediction of non-alcoholic fatty-liver disease and liver fat content by serum molecular lipids. Diabetologia. 2013 Oct;56(10):2266-74.
(2) Zhou Y, et al. Noninvasive detection of nonalcoholic steatohepatitis using clinical markers and circulating levels of lipids and metabolites. Clin Gastroenterol Hepatol. 2016 Oct;14(10):1463-1472.e6

Title
Well B: personalized metabolic profiles for improved health through precision medicine

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Abstract text

During the last few years, the concept of personalized or precision medicine has been spread out to address the problem of noncommunicable diseases (NCDs) such as cardiovascular diseases or diabetes. Precision medicine basically consists of adapting lifestyle (diet and physical activity) to individual needs, taking into account the molecular characteristics (genetic and metabolic) of the patient. Nevertheless, there are still barriers for bringing these technologies closer to people and a shift is needed to make them more patient-centered, reliable, trusted, scalable and sustainable. Well B is a test thought to go beyond these limits.

The test uses a small blood sample from a finger puncture which is collected in a special device by the patient himself. The sample can then be delivered to our facilities where it is analyzed in a LC-MS system. Both the device and the compounds used as biomarkers have been selected due to its stability during the transport, storage and analysis to ensure reliability. The patient also receives access to an own online platform where medical history and data about lifestyle is recorded and analyzed to give a more accurate and personalized recommendations.

Preliminary data shows that these biomarkers correlate with several risk factors, signs and symptoms of NCDs, such as waist circumference, waist-to-hip ratio, body mass index, blood sugar, blood lipids, insulin resistance or hypertension, and that some of the biomarkers can be modified by lifestyle interventions focused on diet and physical activity. This means that the patient might be having different kits to assess his metabolic response to the changes generated by the test advice.

Biomeb is a biotechnology company, a spin-off of the University of Lleida, whose mission is to provide personalized medicine, providing new products and services to society that mean a breakthrough in the prevention, diagnosis and personalized treatment of different pathologies. Our company is founded by PhD from diverse backgrounds in physiology and biochemistry, and our collaborators are experts in healthcare, statistics, bioinformatics, information technologies and software development.

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Title Diagnosis and staging of multiple sclerosis using a combined 'omics approach.
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Abstract text

As there is currently no diagnostic biomarker or biofluid test available for multiple sclerosis (MS), diagnosis relies on the exclusion of other possible conditions mimicking MS. The existence of several demyelinating diseases of the central nervous system (CNS), all with clinical phenotypes overlapping with MS, can make this particularly challenging. We have discovered, using a range of metabolomics techniques (including nuclear magnetic resonance spectroscopy, mass spectrometry, and lipidomics) coupled with multivariate analysis, that the serum metabolite profile is able to diagnose MS with 100% accuracy compared to healthy controls. Furthermore, this metabolite profile discriminates MS from similar CNS diseases which are impossible to separate using clinical features alone; aquaporin-4 (AQP4)-antibody (Ab) and myelin oligodendrocyte glycoprotein (MOG)-Ab diseases with accuracies of 94% and 73% respectively. All three diseases exhibit a unique plasma metabolite pattern which is independent of disease severity, suggesting that metabolomics of blood may be of use in diagnosing patients with clinically overlapping demyelinating diseases. In addition, we have demonstrated that metabolomics can be used to distinguish between the early relapsing remitting (RR) stage and the secondary progressive (SP) stage of MS with high accuracy (87%). The transition from RR to SPMS is extremely subtle and often difficult to identify clinically. The diagnosis of SPMS is only made retrospectively when accrual of clinically apparent disability has occurred, usually 2 to 3 years after the onset of progression. Furthermore, there are currently no recognised clinical criteria or biomarkers that can identify disease progression, particularly at the initial phase. As the paradigm of MS drug development shifts towards preventing disease progression instead of merely reducing relapses, our approach could be a powerful tool to identify early progression when pharmacological intervention could potentially stave off future disability worsening. Indeed, not only have we demonstrated that the metabolite profile is able to identify this subtle change in disease stage using a range of methods, we have been able to identify the transition to SPMS up to one year earlier compared to clinical evaluation in a small cohort of patients at Oxford. Combined, these results highlight the potential of metabolomics analysis of blood for the diagnosis, staging, and monitoring of MS.

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Title
Quantitative targeted analysis of amino acids in Norwegian prostate cancer patients: an association with biomarkers of prostate cancer and inflammation

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Background
We have previously shown that a nutritional intervention with tomato products alone or in combination with selenium and n-3 fatty acids, soy isoflavones, grape/pomegranate juice, and green/black tea lower prostate-specific antigen (PSA) in patients with non-metastatic prostate cancer[1]. As revealed by targeted metabolomics, this study also demonstrated that the nutrition metabolome was largely affected by the intervention. A recent meta-analysis[2] concluded that selected amino acids are related to cancer. Therefore, we used a targeted metabolomics approach of amino acids to study the effect of the diet intervention in the prostate cancer patients.

Methods
We included 74 patients with prostate cancer from the previous study. Before curative treatment, the patients were randomized to nutritional interventions with either 1) tomato products containing 30mg lycopene per day; 2) tomato products plus selenium, omega-3 fatty acids, soy isoflavones, grape/pomegranate juice, and green/black tea; or 3) control diet for three weeks. Plasma samples from baseline and after the intervention were analyzed. Total PSA was determined on the AutoDELFIA automatic immunoassay system and C-reactive protein (CRP) by the accredited (NS-EN ISO/IEC 17025) Dr. V. Fürst Medical Laboratory. Plasma carotenoids were detected using HPLC. A quantitative targeted metabolomics analysis including 26 Sulphur-containing amino acids was run using LC/MS-MS(ABSciex QTRAP5500). An ANOVA adjusted for the baseline was performed to evaluate differences between groups after the dietary intervention. Correlation between parameters was estimated by computing Pearson's correlation coefficient using the delta(t1-t0). Statistical analyses were performed using SPSS 24.

Results
For all patients, we found a significant correlation between the lycopene change and total PSA($r=-0.247$; $p=0.034$) and ornithine($r=0.238$; $p=0.041$). Furthermore, the change in ornithine was inversely correlated with total PSA($r=0-0.311$, $p= 0.007$). Finally, the change in total PSA was correlated with CRP($r=0.301$; $p=0.011$).

Conclusion:
A quantitative targeted metabolomics approach revealed an association between the increase of ornithine and lycopene. Furthermore, ornithine showed to be inversely correlated with PSA, which in turn is associated with the inflammatory biomarker, CRP. In a follow-up study, we will use untargeted metabolomics (Orbitrap LC-MS) to study intervention effects on the global metabolome.

References:
1. Paur, I. *et al.* Tomato-based randomized controlled trial in prostate cancer patients: Effect on PSA. *Clin. Nutr.* 4–11(2016).
2. Goveia, J. *et al.* Meta-analysis of clinical metabolic profiling studies in cancer: challenges and opportunities. *EMBO Mol Med* **8,** 1134–1142(2016).

Title

Persistent alterations in serum lipid profiles prior to gluten intake predicts progression to Celiac disease during early infancy

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Abstract

Celiac disease (CD) is a chronic enteropathy characterized by an autoimmune reaction in the small intestine in genetically susceptible individuals. It is well known that gluten is the required environmental trigger of clinical CD, but the underlying causes for the autoimmune reaction are yet unknown. Herein, we elucidate the early events preceding the clinical disease in a prospective series of children followed-up over 6 years from first exposure to gluten (3-4 months) to recommended gluten free diet after diagnosed with CD. We have applied global lipidomics profiling by UHPLC-MS in 233 plasma samples from the Type 1 Diabetes Prediction and Prevention (DIPP) study (n=23 CD progressors, n=23 controls). The lipidomic profiles revealed that children who later progressed to CD had increased triglycerides (TGs) of low carbon number and double bond count and decrease of phosphatidylcholines already at four months of age. The differences were exacerbated with age but were not observed at birth (cord blood). No significant differences were observed in dietary TGs such as those containing polyunsaturated fatty acids. Our findings suggest that an abnormal lipid absorption may occur already prior to the intake of dietary gluten in clinical CD. Moreover, the specific TGs found elevated in CD progressors may be due to a host response to compromised intake of dietary lipids in the small intestine, leading to the *de novo* synthesis.

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Title

The metabolic pattern could be useful for better understanding of the etiology of cardiovascular diseases.

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Abstract text

Cardiovascular diseases are a leading cause of morbidity and mortality worldwide accounting for 32% of all deaths across EU countries. Estonia exhibits the higher prevalence of the cardiovascular diseases, whereas, half of deaths are caused by circulatory system diseases according to the Estonian Causes of Death Registry. Adverse blood pressure is a major independent risk factor for cardiovascular diseases (CVDs). There are insufficient previous systematic studies on blood metabolomics in human hypertension and CVD.

We profiled the metabolic pattern of serum of patients classified according to International Classification of Diseases (ICD10) with stable ischemic heart disease (IHD) without previous myocardial infarction (code I20), with previous myocardial infarction (I25.2) and the patients with hypertensive heart disease (HHD, code I11). This study was performed in accordance with the Helsinki Declaration and was approved by the Tallinn Medical Research Ethics Committee.

The filtered venous serum from age and gender matched IHD patients ICD10 coded I20 (n=12), I25.2 (n=6), I11 (n=25) and control individuals (n=20) were analyzed using one-dimensional proton nuclear magnetic resonance (1H NMR) spectroscopy. NMR based metabolomics is characterized by (i) high information content, (ii) convenient and non-destructive sample preparation, (iii) reproducible signal intensities, (iv) accurate quantification. These spectra were allocated to metabolic profiling and concentration calibration (Chenomx Inc) followed by statistical analysis using one-way ANOVA and principal component analysis.

We identified from serum spectra about 83 metabolites. The metabolite concentrations are in good accordance with results provided by clinical chemistry reference values, the Human Metabolome Database (HMDB) library and various studies. The major implications found in the serum of the patients is related to the concentration changes of acetylacetate, choline, pyruvate, betaine, formate and alanine, creatine, glycine, histidine, lactate, proline, urea and other biomolecules.

A number of changes of calibrated metabolites are connected to human microbiome according to the given scientific information. Previous studies have revealed a less rich and diverse microbiota in hypertensive compared to control subjects. Interestingly, the altered health conditions might be possibly characterized by altered microbiome.

Chemometrics analysis exhibit a significant difference among the IHD patients, HHD patients and control individuals. These data support that metabolomics approach may be beneficial for the early detection of circulatory diseases, for molecular understanding of particular health condition, and for detection of synergistic pathways engaged in the development of altered health conditions.

Food and Health

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Title
Brain cortex metabolome is altered by high fat diet: potential effect of probiotics in a prepuberal female pig biomedical model.

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Abstract text

Obesity has become a global epidemic. The trend of increasing obesity is also of concern because of its increased comorbidities. This condition leads to low-grade inflammation in several peripheral tissues as well as in central nervous system. Further, neuroinflammation causes intracellular disturbances and exacerbates various stresses such as oxidative stress. Consequently, there is an urgent need to identify molecular markers that will enable early detection of the progressive pathophysiological dysfunctions leading to the neurological co-morbidities and the development of effective therapies.

In this study, metabolomics profiling of brain samples (frontal cortex) from euthanized female pre-puberal pigs (135 days) was performed by UPLC-QTOF-MS, in 4 different dietary groups: i) normo-caloric, ii) high-fat, iii) high-fat supplemented with rice protein hydrolysates and probiotic *B breve* and iv) the same as iii with 2% of omega-3 fatty acids. The obtained data was subjected to multivariate statistical analysis to find metabolites that contribute to the discrimination between those groups. As the arose biomarkers suggested the role of oxidative stress, GC-MS was used to quantify protein oxidative damage. The content of mitochondrial complex I –a modulator of oxidative stress- was assessed by evaluating the amount of several subunits, including (NDUFV2, NDUFS4, NDUFS3, NDUFA9) by Western Blot.

We show that 237 metabolites were significantly affected in the four dietary groups. Among the identified metabolites, some belonged to the family of oxidized polyunsaturated fatty acids generated from arachidonic acid, which were found to be increased in the high-fat group (group ii). Moreover, the pigs in this latter group had higher levels of N(-(malondialdehyde)lysine (MDAL), a protein mark of lipoxidation. The administration of probiotics was capable to revert the effects of a high-fat diet, by partially remodeling the brain metabolome and decreasing the levels of lipoxidative damage, as well as the content of mitochondrial complex I subunits. These results globally suggest that a high-fat diet is capable to modulate the brain metabolome although these effects can be moderated by the administration of probiotics.

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Title
Cord blood metabolites are associated with development of early childhood allergy

Authors (presenting author underlined)
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Abstract text

Allergy is the most common disease among young children, yet causes and mechanisms behind development of childhood allergy remain unclear and controversial. In a small exploratory study comparing allergy rates among children brought up on dairy farms compared to children brought up in the same rural area, but not on farms, we have used GC-MS metabolomics (1) to explore what metabolites in umbilical cord blood are associated with development of allergy at 18 months, 36 months and 9 years. A total of 21 children from farms and 23 children not from farms with available cord blood samples were used in this study, and 10, 6 and 7 children had allergy at ages 18 months, 36 months and 8 years respectively. Subjects who had allergy and later grew out of it were removed from subsequent analyses. Several metabolites differed between allergic and non-allergic children at the different ages assessed, though no metabolites were predictive of allergy at all three ages assessed. This suggests that if imprinting of allergy risk occurs *in utero*, that this differs depending on the type of allergy. Results of note included the non-steroidal anti-inflammatory drug loxoprofen being higher in cord blood of children who were allergic at 36 months and 9 years, and bile acids chenodeoxycholic acid and ursodeoxycholic acid being higher in children who were allergic at 18 and 36 months, but not 9 years. The presence of loxoprofen may suggest genetic predisposition leading to increased maternal use of anti-inflammatory drugs, while differences in bile acids supports earlier work finding that fat intake, fatty acid composition in umbilical cord blood may be related to development of allergy (2). A major limitation of this study is the low number of subjects, and for robust modelling of the role of metabolism/metabolites in the development of allergy, much larger study cohorts are required.

(1) Savolainen O, et al. A simultaneous metabolic profiling and quantitative multimetabolite metabolomic method for human plasma using gas-chromatography tandem mass spectrometry. J Proteom Res. 2016 Jan 4; 15(1):259-65.
(2) Barman M, et al. High levels of both n-3 and n-6 long-chain polyunsaturated fatty acids in cord serum phospholipids predict allergy development. PLoS One. 2013 Jul 10;8(7):e67920.

1st Nordic Metabolomics Conference
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Title
Network analysis reveals heterogeneous redox responses in hepatocellular carcinoma patients

Authors (presenting author <u>underlined</u>)
<u>Rui Benfeitas</u>[1], Gholamreza Bidkhori[1], Bani Mukhopadhyay[2], Martina Klevstig[3], Muhammad Arif[1], Cheng Zhang[1], Sunjae Lee[1], Resat Cinar[2], Jens Nielsen[4], Mathias Uhlen[1], Jan Boren[3], George Kunos[2], Adil Mardinoglu[1]

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Abstract text

Redox metabolism is often considered a potential target for cancer treatment, but a systematic examination of redox responses in hepatocellular carcinoma (HCC) is missing. Here, we employed systems biology approaches integrating omics data to reveal key roles of genes associated with redox metabolism in HCC. We found that several redox genes, including 25 novel potential prognostic markers, are significantly co-expressed with liver-specific genes and genes associated with immunity and inflammation. HCC tumors display antagonistic behavior in redox responses. The two HCC groups are associated with altered fatty acid, amino acid, drug and hormone metabolism, differentiation, proliferation, and NADPH-independent vs -dependent antioxidant defense. Redox behavior varies with tumor subtype and progression, affecting patient survival. These antagonistic responses are also displayed at the metabolomic and protein level, and were validated in several independent cohorts. Experiments in mice reinforce the observed differential redox behavior, associated with hypoxic features of the two redox gene groups. Our integrative approaches highlighted mechanistic differences among tumors and identified a novel survival signature and potential subgroup-specific therapeutic targets for HCC treatment.

Title
Mycotoxin-database on Collision-Cross-Sections derived by TWIMS-HRMS analysis.

Authors (presenting author underlined)
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Abstract text

Introduction:
Accurate identification and quantification of toxic fungal metabolites (=mycotoxins) are the essential requirements for reliable assessments of food safety. Recently designed and powerful combination of Ultra-high-performance-chromatography (UHPLC), Ion-Mobility Spectrometry (IMS) and high-resolution-mass-spectrometer (HRMS) meet those requirements and can provide additional structural information by means of Collision Cross Sections (CCS), that have not been documented for mycotoxins, yet.
Method:
We implemented a Travelling Wave-Ion-Mobility-(TWIMS)-routine into our (UHPLC-)HRMS analytical workflow, not only to identify mycotoxins by retention times and *m/z*-values but in addition to provide $^{TW}CCS_{N2}$ values of mycotoxins from different matrices.
Therefore we extracted and analysed (bio-)synthesized mycotoxins from standard-solutions, four complex cereal matrices (wheat, maize, rye and malt) spiked with our mycotoxins ($2mg \cdot L^{-1}$) as well as from 10 naturally incurred samples including cereals/cereal-based products and a reference material, respectively.
Results:
For the first time, a library of 106 $^{TW}CCS_{N2}$ values of 61 mycotoxins (ranged from 130Å - 290Å) was created, including regulated as well as not yet legislated mycotoxins. Findings have given proof of the robustness of our method by very few interferences from different matrices (RSD <0.9%) and high reproducibility (RSD <2%) across different instrumental conditions. Unexpectedly, we identified 14 mycotoxins adduct species formed especially with smaller molecules (300-500Th) that seem to depend on the presence of particular ions in sample extracts.
Conclusion:
Through this we are convinced that our method and library will provide additional information to fungal metabolite research and will support as well as inspire future investigations on metabolic and analytical aspects regarding mycotoxins and food safety applications, respectively.

(1) Righetti L, Bergmann A, Galaverna G, Rolfsson O, Paglia G, Dall'Asta C. Ion mobility-derived collision cross section database: Application to mycotoxin analysis. Analytica Chimica Acta. 2018 Jul 19;1014:50-57.

P49 **Title**
Effect of healthy Nordic diet on plasma and urine metabolic profiles

Authors (presenting author underlined)
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Abstract text

The beneficial effects of healthy Nordic diet (HND) on cardiometabolic health have been recently shown by a randomized dietary intervention (Sysdiet; 6 centres across Nordic countries)[1], yet underlying mechanisms are not known. Therefore, the aim of this study is to (i) asses a panel of dietary intake biomarkers defining HND and (ii) identify the effect markers and interpret the biochemical mechanisms in relation to beneficial effects of HND using plasma and urine metabolic profiles from Sysdiet dietary intervention. 159 subjects with measures of the metabolic syndrome were randomised to HND or the control diet (average Nordic diet, AND) for 18-24 weeks. Healthy diet included whole-grain products, berries, fruits and vegetables, rapeseed oil, three fish meals per week and low-fat dairy products whereas control diet compromised of an average Nordic diet. Metabolic profiles of fasting plasma and urine at baseline and end of the intervention periods were acquired by UPLC-QTOF. PLSDA achieved to discriminate the diets with AUC of 0.82 and 0.91 for plasma and urine, respectively. Metabolic profiles of HND reflected consumed food groups *i.e.* whole grain intake is represented with alkylresorcinols (and their metabolites), furan fatty acid metabolites reflected fish intake and others were related to berry consumption. Phospholipids containing PUFA and reflecting fish intake (*i.e.* LPC(20:5)) were increased for HND whereas LPC(22:4), associated with animal fat intake, was attributed to control diet. In relation to metabolic effects of HND, an increase in specific acyl-carnitines suggested improved fatty acid metabolism.

In conclusion plasma and urine metabolite profiles were predictive of dietary patterns and reflected good compliance while indicating effects of potential health benefit of HND, including changes in fat metabolism.

1. Uusitupa, M. *et al.* Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome - a randomized study (SYSDIET). *J. Intern. Med.* **274,** 52–66 (2013).

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Title
The use of metabolomics in cheese flavor

Authors
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Abstract text

The flavor of all cheese develops during the ripening process, when complex biochemical changes take place. The ripening and associated flavor development of hard cheeses, such as cheddar, is well defined but the same processes are not well studied in soft cheeses. This makes it difficult for manufactures to produce such cheese with uniform flavor; this can hinder mass production of these products. Similarly, it is hard to isolate processes that cause 'off flavors' or predict when they will occur. Such problems often only become apparent when the cheese ripening process is finished; since this can take months (or even years) these issues can have a significant, negative economic impact. Metabolomics (the scientific study of the small bio-molecules present within biological systems) shows great promise for the study of cheese ripening as there are many small molecules contributing to the flavor profile of cheese and the highly interconnected nature of the taste also means that important information is most likely to be found in correlation patterns as opposed to individual signals. In this study we have used Solid Phase Micro-Extraction and Gas Chromatography Mass Spectroscopy based Metabolomics to analyze flavor development during the ripening of Australia Camembert cheese in association with Ld&D Foods Pty. We present the optimized analytical methodology and show how it has been used to generate new knowledge about the volatile compounds that contribute to the flavor of Australian cheese.

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Title
Metabolic profiles of mothers, fathers and their newborn infants

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Abstract text

Background: Metabolite concentrations in humans are a result of an interaction between genetic and environmental factors. It is unclear what relationship there is between the metabolic profiles (metabolome) of different members of the same family.

Aim: To study if the metabolome of the newborn infant is influenced by the parents. Also, to examine differences in the metabolic profile between arterial and venous umbilical cord blood in relation to the metabolic profile of the mother.

Method: Blood samples were collected from 52 families participating in the NICE birth-cohort. Samples from the mothers at delivery, samples from the fathers, and arterial, venous and mixed umbilical cord blood plasma from the newborn babies were analysed with GC-MS/MS, measuring a combination of untargeted, selected ion- and multiple reaction monitoring measurements during a single run (1). Multivariate data analysis tools such as PCA, PLS, PLS-DA and RF as well as ANOVA decomposition and Multilevel PLS and RF algorithms, were used to check if there were any systematic differences between all the different groups. Univariate methods such as Welch's t-test and paired t-tests were used as a complement to the multivariate statistics.

Results: Samples from mothers generally contained high amounts of α-tocopherol and fatty acids, mainly oleic-, linoleic- and linolenic acid, compared to paternal and infant samples. Venous, arterial and mixed cord serum samples contained high amount of amino acids, mainly glycylvaline, lysine, phenylalanine and tryptophan. Arterial blood samples contained more mono- and disaccharides such as deoxy-galactose, glucose, sorbose and galactose while venous blood samples contained more organic acids including α-ketoglutaric acid and glutamic acid.

Conclusions: Differences between mothers and newborns reflect differences in catabolic/anabolic states. Arterial and venous differences may indicate what substrates are being preferentially used by the newborn baby.

(1) Savolainen, O. I., Sandberg, A. S., & Ross, A. B. (2015). A simultaneous metabolic profiling and quantitative multimetabolite metabolomic method for human plasma using gas-chromatography tandem mass spectrometry. Journal of proteome research, 15(1), 259-265..

1st Nordic Metabolomics Conference
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Title
Non-targeted LC-MS metabolomics as an approach for determining vitamin B requirement of mink

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Abstract text

Correct determination of vitamin B requirement in animal and man is demanding due to analytical challenges for proper quantitative measurements in feed and appropriate metabolic markers in the animal. Domestic mink is no exception, and the vitamin B complex is traditionally added to mink feed in amounts up to 10-80 times above indicated requirements. Surplus of the water-soluble vitamin B complex is rapidly excreted in the urine. The aim of the present project was to use a non-targeted LC-MS metabolomics approach to study different vitamin B and metabolites thereof and other relevant metabolites excreted in the urine of mink fed diets with different amounts of the vitamin B complex. Two studies were performed in the growing period (July-November). In study 1, four levels of vitamin B complex was used: no addition of vitamin B, addition of 50% of the recommended level, addition of the recommended level, and addition of the recommended level plus a supplement of liquid vitamin B. In study 2, minks were fed either a control diet with the recommended level of vitamins or the same diet without addition of vitamins. Urine samples were collected from 10 males per group in September in study 1 and from 15 males per group in September and November in study 2. The samples were prepared for non-targeted LC-MS metabolomics and data were acquired on a UHPLC (Dionex, Sunnyvale, CA, USA) coupled to an Impact HD mass spectrometer (Bruker Daltonics, Bremen, Germany). The data were preprocessed using Bruker Compass DataAnalysis 4.2 and ProfileAnalysis 2.1, Principal Components Analysis (PCA) and Partial Least Squares regression (PLS) were performed using LatentiX 2.12. In study 1, PCA scores plots revealed that the metabolomics pattern for mink fed without vitamin B supplementation separated from the three other groups, which on the other hand were indistinguishable. PLS scores plots of the data in study 2 showed that the samples grouped according to both diet and sampling date. In study 1, the excretion of riboflavin (vitamin B_2), pantothenic acid (vitamin B_5), and pyridoxine (vitamin B_6) increased with increasing amount of vitamin B complex in the diets. Furthermore, the excretion of N-Methyl-2-pyridone-5-carboxamide, 1-methylnicotinamid, 4-pyridoxic acid, and 5-methyl-tetrahydrofolate showed that vitamin B_3, B_6 and B_9 were present in excess too. Study 2 confirmed that these B vitamins were in excess also in November. The findings of the present studies suggest that today's vitamin B complex supplementation to mink may be lowered.

1st Nordic Metabolomics Conference
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ABSTRACT

Title
Urinary phenolic metabolites in healthy men after consumption of acylated anthocyanins

Authors (presenting author <u>underlined</u>)
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Abstract text

The polyphenolic natural colorants of fruits and berries, the anthocyanins, are shown to have beneficial health effects. For example, anthocyanins or anthocyanin-rich foods reduce the risk of insulin resistance, and attenuate postprandial glycemia and insulinemia. (1–3) As anthocyanins are rapidly degraded after a meal, their metabolites may have a valuable impact on human health. (4)

Most of the recent studies on anthocyanin metabolism have focused mainly on the phenolic metabolites after a berry meal, such as raspberries (5) and blueberries (6). After absorption from the small intestine, anthocyanins are metabolized into glucuronide and sulfate conjugates. Gut microbiota catabolizes anthocyanins further leading to phenolic acids. (5–7) However, knowledge of the metabolites formed after a meal containing acylated anthocyanins is still scarce.

The objective of this study was to analyse the anthocyanins, anthocyanin conjugates and phenolic metabolites excreted into the urine of healthy men after a meal with and without added acylated anthocyanins in a single-blinded, cross-over trial. The metabolites were purified from the urine matrix by solid-phase extraction, and putative metabolites were screened using LC-MS in multiple reaction monitoring mode. These results will provide novel information on the metabolism of acylated petunidin, peonidin and malvidin glycosides.

(1) Li D, et al. Purified anthocyanin supplementation reduces dyslipidemia, enhances antioxidant capacity, and prevents insulin resistance in diabetic patients. J Nutr. 2015 Apr;145(4):742-8.
(2) Linderborg KM, et al. Comparison of the postprandial effects of purple-fleshed and yellow-fleshed potatoes in healthy males with chemical characterization of the potato meals. Int J Food Sci Nutr. 2015 Aug;67(5):581-91.
(3) Törrönen R, et al. Berries modify the postprandial plasma glucose response to sucrose in healthy subjects. Br J Nutr. 2010 Apr;103(8):1094-7.
(4) deFerrars RM, et al. Phenolic metabolites of anthocyanins following a dietary intervention study in post-menopausal women. Mol Nutr Food Res. 2014 Mar;58(3):490-502.
(5) Zhang X, et al. An exploratory study of red raspberry (*Rubus idaeus* L.) (poly)phenols/metabolites in human biological samples. Food Funct. 2018 Feb 21;9(2):806-18.
(6) Feliciano RP, et al. Plasma and urinary phenolic profiles after acute and repetitive intake of wild blueberry. Molecules. 2016 Aug 25;21(9), 1120.
(7) Czank C, et al. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. Am J Clin Nutr. 2013 May;97(5):995-1003.

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Metabolite profiles of habitual diet in serum- a metabolomics approach to evaluate intake of meat or foods of animal origin by NMR-analysis of serum samples

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To prove associations and causation between diet and health, objective and reliable methods are needed to measure dietary exposure. This study aimed to investigate if ^{1}H-NMR analysis of serum samples could be used as an objective method to discriminate vegan, vegetarian or omnivore habitual diets. Specifically, the aim was to find a pattern of metabolites that separated meat-consumers from non-meat consumers and vegans from non-vegans.

Healthy volunteers (n=120; age 19-57) were recruited by advertisement for individuals with habitual vegan-, vegetarian- or omnivore diets. Data on clinical phenotype, body composition, a short lifestyle questionnaire including a food frequency questionnaire (FFQ) and a four-day weighed-food diary were collected. Serum samples were analyzed for creatinine, C-reactive protein, glucose, hemoglobin, vitamin B12, folate and calcium and for metabolites using 1H NMR spectroscopy. NMR data were non-normalized, UV-scaled and analyzed with multivariate data analysis (Projections to Latent Structures with Discriminant Analysis, PLS-DA and Orthogonal Projections to Latent Structures with Discriminant Analysis, OPLS-DA). Metabolites were identified using 1D 1H NMR and 2D HSQC spectra. Even though many metabolites differ in concentration between men and women, and by other factors such as age, body mass index and body composition, it was possible to identify discriminating patterns in relation to intake of both meat and products of animal origin. Branched-amino acids, creatinine, creatine, glutamine, glycine and trimethylamine were discriminating metabolites. To conclude, ^{1}H-NMR analysis of fasting serum samples can be used as an objective method to evaluate intake of meat and other foods of animal source.

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ABSTRACT (template)

Title

Postprandial response to the oral-fat tolerance test (OFTT) on plasma metabolomics profile

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Abstract text

It is widely known that the postprandial response to a meal depends on many factors and involves multiple processes that include energy storage and metabolic switch in several organs such as liver, muscle and adipose tissue, accompanied by several compensating processes such as inflammation and oxidative stress (1). The oral-fat tolerance test (OFTT), which is based on an oral fat load administered to fasting people, has been widely used to assess the postprandial lipemia. Alterations in the physiological response to the OFTT can aid in the study of metabolic disorders.

The present research is focused on detecting postprandial alterations in the level of plasma metabolites after the OFTT, which consisted of a weight-adjusted meal (0.7 g fat and 5 mg cholesterol per kg body weight) with 12% saturated fatty acids (SFAs), 10% polyunsaturated fatty acids (PUFAs), 43% monounsaturated fatty acids (MUFAs), 10% protein, and 25% carbohydrates. Plasma samples were collected from 200 patients, involved in the CORDIOPREV study, just before and four hours after the OFTT. Collected plasma samples were analyzed by LC–QTOF MS/MS and GC–TOF/MS in three different batches including 52, 116 and 32 individuals, respectively.

A total number of 365 metabolites were tentatively identified by combination of both analytical techniques. The repeated-measures ANOVA led to the identification of 49 metabolites significantly altered ($p<0.05$) due to the OFTT in all batches. The significantly altered compounds were fatty acids and derivatives (19), bile acids and derivatives (13), carnitines (4), amino acids and analogues (2), benzene and substituted derivatives (2), carboxylic acids (2), phospholipids (2), steroids (2), endocannabinoids (1), glycerolipids (1) and prenol lipids (1). Special attention was paid to fatty acids, their derivatives and bile acids since these families comprised the 65% of significant metabolites. Alterations in the fatty acids profile were in concordance with the composition of the OFTT meal. On the other hand, the pathways for the biosynthesis of primary and secondary bile acids resulted to be affected by the OFTT meal with preferred production of glycocholic acid versus that of taurocholic acid. The identification of postprandial metabolic alterations occurring during the OFTT can lead to interpret deviations associated to metabolic disorders.

FADS1 **genotype modifies metabolic response to dietary linoleic acid in genotype based clinical trial**

Authors
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Abstract text

The genetic variation on *FADS* gene cluster is associated with differences in fasting cholesterol, insulin and glucose levels. Delta-5 desaturase (D5D) enzyme, encoded by *FADS1* gene, is one of the key enzymes which determines the rate of endogenous formation of long-chain polyunsaturated fatty acids (PUFAs). The homozygous minor C allele on *FADS1* rs174550 decreases the D5D activity. However, there are no studies investigating the effect of this gene variant on plasma metabolic profile overall. The present study examined the effect of *FADS1* rs174550 polymorphism (26 men with TT and 33 men with CC genotypes) on metabolism in a 4 week controlled dietary intervention. Participants consumed 30-50 ml/day linoleic acid (LA) rich sunflower oil, which provided approximately 6 E% of LA, without other changes in their habitual diets. We aimed to see differences in inflammatory markers and metabolic responses between genotypes. The fasting plasma EDTA samples were analyzed by non-targeted metabolite profiling approach utilizing liquid chromatography time-of-flight mass spectrometry with reverse phase and hydrophilic interaction chromatography. The differences in metabolic profile responses were investigated with linear regression model. At the baseline genotypes showed differences in the levels of various phosphatidylcholine (PC) and lysophosphatidylcholine (LysoPC) species. TT genotype was generally associated with higher levels of lipid species, which contain more unsaturated forms of PUFAs, such as LysoPC(20:4) and LysoPC(20:5). After a 4-week LA rich diet the TT genotype showed tendency (P< 0.01) towards decreased levels of cortisol and increase in monoacylglycerol 18:1 and PC(16:0/22:5) levels. CC genotype showed opposite effect on these metabolites. Also, genotype specific response on a serum fasting high sensitive CRP and plasma glucose concentrations were seen. In conclusion, *FADS1* rs174550 genotype modified the responses in metabolism and the inflammatory processes in a LA rich diet. Further studies with bigger sample size, and probably also with n-3 fatty acid enriched diets, are needed to confirm the findings and to elucidate the role of n-6 and n-3 PUFAs on metabolic responses in persons with different *FADS1* genotypes.

Title

IDENTIFICATION OF IBS METABOTYPES BASED ON PHYSIOLOGICAL RESPONSES TO FOODS

Authors (presenting author <u>underlined</u>)
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Abstract text

Irritable bowel syndrome (IBS) is a condition characterized by abdominal pain, bloating, constipation, diarrhea and gas and affects up to 15% of the Western population. In many individuals with IBS, symptoms can be triggered by foods, such as FODMAPs (easily fermentable dietary fiber containing Fermentable Oligosaccharides, Disaccharides, Monosaccharides, and Polyols). Some individuals with IBS may also benefit from a gluten-free diet. Current subtypes of IBS are based on symptoms (constipation, diarrhea, and mixed), rather than mechanistic differences. Another promising approach for identifying IBS subtypes is based on grouping individuals into similar metabolic phenotypes, i.e. metabotypes, that share similarities in metabolism and metabolic regulation in response to specific foods. Health and wellbeing could potentially be improved by personalized treatment through tailoring diet to subjects with different IBS subtypes.

To investigate this hypothesis, we will conduct an intervention study on subjects with IBS and identify specific food susceptibilities based on metabolic phenotype (metabotype). In total, 120 women and men with moderate to severe IBS will be recruited. Gluten intolerance, other gastrointestinal disease and abdominal surgery will constitute exclusion criteria. The study will be performed in a double-blind, randomized, placebo-controlled cross-over study design. Study participants will receive three 1-week diets with additions of either FODMAPs, gluten or an inert control with 1-week washout in-between. IBS metabotypes will be identified by integrative multivariate analysis of molecular phenotype data from metabolomics and microbiota measurements combined with data on bowel habits and stomach discomfort. Study participants will also be subjected to a cocktail provocation containing FODMAPs and gluten to develop a rapid diagnostic test based on identified plasma metabolomic biomarkers of IBS metabotypes.

Title
Metabotypes Determining Cardiometabolic Risk and Personalized Diet Strategies to Improve Health

Authors (presenting author underlined)
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Abstract text
Diet is a major modifiable risk factor of metabolic diseases such as type 2 diabetes and cardiovascular disease (CVD). However, evidence is accumulating that there may be inter-individual differences in the response to foods and dietary changes. The gut microbiota plays a crucial role in human metabolism and has recently been shown to determine the health response to diet within an individual. Thus, the interaction between diet and microbiota seems to be of high importance for the metabolic effects of dietary exposures. In this study we seek to identify metabotypes and unique biomarkers that can guide personalization of dietary interventions for adults at high risk for CVD.

We will assess the plasma metabolome, the gut microbiome and CVD-associated lifestyle factors of 750 adults from a sub-cohort of the Danish Diet Cancer and Health- Next Generation study. To ensure high quality metabolomics data with minimal undesired technical variability, we will employ the established in-house work flow for large-scale LC-qTOF-MS analysis. To decipher different metabotypes from the metabolomics data, we will continue to develop novel statistical tools that will also allow us to assess the contributions of metabotype determinants from the other available multivariate data sources (e.g., gut microbial data). Following metabotype identification, 80 individuals matched to the defined metabotypes will be recruited and undergo 6-week long cross-over dietary interventions to evaluate the responses to fermentable and nonfermentable cereal fiber. Metabolomics and gut microbial analysis will be used to monitor shifts in the CVD phenotype throughout the intervention, as well as to evaluate the differential response for potential responders and non-responders.

In conclusion, the concept of monitoring diet-microbiota interactions to determine the risk of developing metabolic disease is novel, yet grounded on extensive research. Our aim is that our collaborative effort ultimately will provide evidence-based dietary recommendations, directed towards distinct groups of individuals, that will lead to effective and sustainable health improvements, preventing CVD.

1st Nordic Metabolomics Conference
Örebro, August 26-28, 2018

ABSTRACT

Title

Differential Response in Postprandial Plasma Metabolomics with Pre-Meal Whey Protein Intervention in Subjects with Metabolic Syndrome - PREMEAL 1

Authors (presenting author <u>underlined</u>)
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Abstract text
Aim: Effect of a pre-meal intake of whey protein (WP) on lipid and amino acid metabolism is unclear. We aim to explore the distinctive plasma metabolites of pre-meal intake of WP in subjects with metabolic syndrome.

Method: The PREMEAL-1 study is an acute, randomized, cross-over study. Twenty adult subjects with metabolic syndrome received 0, 10 or 20 g of WP each test day, -15 min prior to a standardized fat-rich breakfast. Blood samples were drawn at -10, 0, 120, 240 and 360 min. Plasma samples were analyzed by UPLC-ESI-Q-TOF-MS. Partial least squares discriminant analysis (PLS-DA) was performed for each time point. For structural elucidation of the selected features, MS/MS experiments were performed at 10, 20 and 30 eV. Results were compared to previously run standards, an in-house database as well as in-silico and experimental fragmentation databases.

Results: WP intervention is related to higher plasma response of several amino-acids (valine, methionine, tyrosine, leucine, isoleucine) and the cyclic dipeptide, cyclo(alanylvalyl), and a lower plasma level of LysoPC (16:0) and PC(16:1(9Z)/16:0) / PC(16:0/16:1(9Z)) at the time point 0, before the main meal. WP intervention altered plasma acyl carnitines, amino acids and several LysoPEs and LysoPCs in the postprandial state. Pre-meal WP intake resulted in higher propionyl carnitine, pentanoyl carnitine, tryptophan, L-gamma-glutamyl-L-leucine levels and lower palmitoyl carnitine, betaine, LysoPE(18:1), LysoPC(18:2) and 3-hydroxyhexadecanoic acid levels in plasma at time point 120 min.

Conclusion: Pre-meal intake of WP alters postprandial disposition of branched-chain and some other amino acids as well as several distinct phospholipids and acyl-carnitines.

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ABSTRACT

Title
Mediterranean diet changes the intestinal and systemic metabolome in overweight/obese adults

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Abstract text

Diet-induced effects on the intestinal and systemic metabolome upon adherence to the Mediterranean diet (Med-D) may be beneficial for maintaining cardiovascular health. To investigate the effects of the Med-D compared to habitual diet on the intestinal and systemic metabolome, we conducted a randomized controlled parallel trial in 82 overweight or obese men and women with no known history of cardiovascular disease. Each subject in the Med-D group was assigned a personalized diet prepared on the basis of his/her habitual diet. Energy values and whole macronutrient composition were unchanged during the Med-D intervention. Feces, urine and blood samples were collected from all participants at baseline, after 4 and 8 weeks intervention. The samples were subjected to untargeted metabolomics using ultra-performance liquid chromatography time-of-flight mass spectrometry (UPLC-MS). Among the metabolites changing with the Med-D, we identified several food biomarkers confirming compliance including reduced levels of carnitine (reflecting reduced meat intake), increased levels of alkylresorcinols (reflecting increased wholegrain intake), increased levels of Urolithin A-3-O-glucuronide (reflecting increased nut intake). Furthermore, the Med-D resulted in reduced levels of the host-microbial co-metabolite p-cresol-sulfate (reflecting reduced colonic proteolysis) as well as changes in the primary bile acid, chenodeoxycholic acid-3-sulfate (suggesting altered bile acid biosynthesis and/or excretion), and reduced acyl-carnitines (possibly reflecting reduced beta-oxidation). Finally, the Med-D resulted in a changed tryptophan metabolism as reflected by alterations in both microbially derived tryptophan catabolites as well as in host tryptophan metabolites. Collectively, these alterations confirm that a Med-D changes the intestinal and systemic metabolome by changing circulating levels of food biomarkers, microbial metabolites and host metabolites.

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ABSTRACT

Title
Evaluation of treatment progress in tuberculosis patients based on their serum metabolite profiles

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Abstract text

Background: tuberculosis (TB) is a leading cause of mortality from a single infection in the world. Multidrug-resistant (MDR) TB is an emerging problem including WHO European Region. In 2014, Ukraine alone had estimated 13 000 (range 12 000-14000) MDR-TB cases while treatment failure was over 70%. Treatment of TB and MDR-TB takes from 6 to 18 months. The tools for evaluation of the treatment progress are limited at present. Bacterial cultures take long time while staining and PCR depend a lot on the sample quality and lab skills of the staff. Here, we evaluate a possibility to monitor treatment progress through metabolite signatures from serum samples of TB patients during treatment. Recent studies in the field of metabolomics revealed that many diseases including TB can be accurately identified by a panel of certain metabolites that reflect the physiological state of the organism and/or host-pathogen interaction.
Methods: a time series (at admission to the hospitals, after one month of treatment and after two months of treatment) of serum sample from TB patients during treatment was collected and analyzed with gas chromatography/mass spectrometry. Spectra were processed and identified through comparison with in-house metabolite library (Swedish Metabolomics Centre) and multivariate analysis (SIMCA, Umetrics) was applied to identify metabolite patterns in groups of patients with good and poor treatment progress (based on clinical observations, chest X-ray and sputum culture).
Results: Among over 100 measured metabolites, we identified those characteristic of good treatment progress 1 month after treatment onset. A valid model for separation of good and poor treatment progress was based on 12 metabolites. One part of these metabolites was presumably reflecting inflammatory response of the host while the other part belonged to pathogen.
Conclusions: our results indicate that there is a pool of serum metabolites that can be potentially used for stratification of the TB patients according to treatment progress.

Title

Quantification of trimethylated compounds from diet intervention samples using liquid chromatography with triple quadrupole mass-spectrometric detection

Authors

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Abstract text

Diets including whole grains have been associated with reduced risk of many chronic diseases, including cardiovascular disease, total cancer (1) and type 2 diabetes (2). In our recent metabolomics study we identified a set of novel trimethylated compounds that were increased in human plasma as a result of dietary intake of whole grains and were associated with biomarkers of glucose metabolism (3). However, as many of these identified compounds are hardly reported in mammals, their role in the metabolic system is mostly unknown. To obtain a more precise picture of their concentrations and associations with diet and physiological biomarkers, a quantitative method using liquid chromatography combined with triple quadrupole mass-spectrometric detection (HPLC-QQQ-MS/MS) was developed comprising a range of trimethylated metabolites, including the newly identified ones as well as more common compounds such as glycine betaine and proline betaine. The calibration and internal standards were either synthesized or purchased, and mass-spectrometric parameters were tested and combined with the chromatographic separation system. The established method was applied for analyzing plasma samples from the SYSDIET intervention study (4) with the emphasis on the effects of a healthy Nordic diet including whole-grain products, local fruits, vegetables, berries and fish.

All together 327 plasma samples from SYSDIET were analyzed before and after the intervention lasting for 18 to 24 weeks. Concentrations of the measured compounds ranged from several thousands of ng/ml of l-carnitine and glycine betaine to a few ng/ml or less among uncommon compounds like alanine betaine and phenylalanine betaine. Healthy diet increased the concentrations of pipecolic acid betaine in plasma ($p < 0.001$) when compared to the control diet. In addition, healthy diet increased trimethylamine N-oxide (TMAO) concentrations while both diets decreased proline betaine concentrations. Further studies of the association of the compounds with the diet and physiological biomarkers are currently in progress.

(1) Aune, et al. Whole grain consumption and risk of cardiovascular disease, cancer, and all cause and cause specific mortality: systematic review and dose-response meta-analysis of prospective studies. BMJ. 2016 Jun 14;353:i2716.

(2) de Munter, et al. Whole grain, bran, and germ intake and risk of type 2 diabetes: a prospective cohort study and systematic review. PLoS Med. 2007 Aug;4(8):e261.

(3) Kärkkäinen, et al. Diets rich in whole grains increase levels of betainized compounds associated with glucose metabolism. Submitted.

(4) Uusitupa, et al. Effects of an isocaloric healthy Nordic diet on insulin sensitivity, lipid profile and inflammation markers in metabolic syndrome - a randomized study (SYSDIET). J Intern Med. 2013 Jul;274(1):52-66.

Using metabolic profiling to explore molecular effects of replacing saturated fat with polyunsaturated fat- a randomized controlled dietary intervention study

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Replacing saturated fatty acids (SFA) with polyunsaturated fatty acids (PUFA) reduces the plasma LDL cholesterol (LDL-C) and has been linked with reduced risk of cardiovascular disease (CVD). The aim of the present study was to gain further understanding of the molecular effects of replacing SFA with PUFA. Recently we conducted a double blind randomized controlled trial replacing SFA with PUFA among healthy subjects with moderate hypercholesterolemia (n=99) (1). In the present sub-study, we performed a comprehensive metabolic profiling using multiple platforms (both NMR and LC-MS technology). A large number of lipid subclasses, and myristoyl- and palmitoylcarnitines, tryptophan and kynurenine were reduced when SFA was replaced with PUFA in the diet. In contrast, bile acids, certain ketone bodies and certain amino acids were increased by the intervention. Branched-chain amino acids, the gut flora metabolite trimetylamineoxide and neopterine, a marker of monocyte activation, were unaffected. Replacement of SFA with PUFA in the diet affected metabolites involved in key metabolic events but had no effect on markers of monocyte activation. Applying metabolomics in randomized controlled dietary intervention trials hold the potential to extend our knowledge of biological and molecular effects of dietary fat quality.

(1) Ulven SM, et al. Exchanging a few commercial, regularly consumed food items with improved fat quality reduces total cholesterol and LDL-cholesterol: a double-blind, randomised controlled trial. Br J Nutr. 2016, Oct;116(8):1383-1393.

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Title

Large-scale Metabolomic Profiling for Precision Medicine and Nutrition-A research on relations among dietary exposures, bone phenotypes and metabolomics

Authors

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Abstract text

In precision medicine and nutrition, identification of sub-groups for tailored prevention and treatment of diseases and their preconditions are key elements. The rapid development of high-throughput bioanalytical methods enables detailed OMICs-analysis of human biological samples. To date, precision strategies have mostly been based on genomics approaches and to some extent proteomics. Metabolomics, i.e. the study of substrates and products of metabolism and other small molecules from external exposures in biological samples, has largely been underexplored in this regard. Untargeted metabolomic profiling can provide a snapshot of the current metabolic state in a biological system and reflect host related processes as well as the activity of microbiota and other exposome domains such as diet, and their interactions. The metabolic trait is an intermediate phenotype that links the genome, proteome and exposome to the clinical endpoints. This Ph.D. program will focus on utilization of metabolomics research in precision medicine and nutrition of different bone phenotypes (i.e. metabotypes): To improve early detection and prediction of bone diseases and; To develop strategies for improved nutrition adapted to metabotypes. We will use data and samples (plasma and adipose tissue biopsies) from Swedish Mammography Cohort–Clinical (SMCC; n=5022), which is a sub-cohort of the large prospective Swedish Mammography Cohort (n=61 433). A unique combination of two liquid chromatography-quadrupole time-of-flight mass spectrometers (LC-qTOF-MS) operated in four modes will be used to analyze the large-scale SMCC sample set. Novel in-house algorithms and R packages (BatchCorr, MUVR, SuperDISCO, etc.) recently developed by the research team will be applied for data management and multivariate analysis. Specifically, the program is aimed to identify new biomarkers for early diagnosis of osteoporosis, to assess dietary exposures using metabolomics and to assess vitamin D stores influenced by multiple factors (diet, UV radiation, skin type, etc.) in adipose tissue.

Title

Food intake biomarkers for tubers

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Abstract text

Background: Tubers are important crops as well as staple foods in human nutrition. Among tubers, the potato in particular has been investigated for its health effects. However, except for its contribution to energy and effects related to resistant starch the role of potatoes and other tubers in human health is still debated. In order to establish firm evidence for the health effects of dietary tubers and processed tuber products, it is essential to assess total intake accurately. The dietary assessment in most studies relies mainly on self-reporting and may give imprecise quantitative information on dietary intakes. Food intake biomarkers are useful objective means to assess intake of specific foods or may be used as an additional measure to calibrate the measurement error in dietary reports.

Method: We conducted a systematic biomarker review for tubers according to the BFIRev standardized protocols for review. Here, intake biomarkers for common tubers, including potatoes and heated potato products, sweet potato, cassava, yam, and Jerusalem artichoke are reviewed and validated.

Results: Candidate food intake biomarkers for heated potato product intake include α-chaconine, α-solanine and solanidine; less evidence is available to indicate peonidin 3-caffeoylsophoroside-5-glucoside and cyanidin 3-caffeoylsophoroside-5-glucoside having high potential specificity for purple sweet potato intake; linamarin may in addition be considered a putative food intake biomarker for cassava. Other tubers also contain toxic glycosides or common contaminants as characteristic components but their putative use as intake biomarkers is not well documented. Alkyl pyrazines, acrylamide and acrolein are formed during cooking of heated potato products while this has not yet been investigated for other tubers; these markers may not be specific only to heated potato but measurements of these compounds in blood or urine may be combined with more specific markers of the heated products, e.g. with glycoalkaloids to assess heated potato products consumption.

Conclusion: We conclude that candidate urine or blood biomarkers exist for potato, heated potato, sweet potato and cassava.

Perspective: Further studies are needed to assess the specificity, robustness, reliability and analytical performance for the candidate tuber intake biomarkers identified in this review. We have conducted a four-ways cross-over meal study to find markers of differently heat-processed potato products. Preliminary results will be presented in light of this biomarker review.

Environmental and plant metabolomics

1st Nordic Metabolomics Conference - Örebro, August 26-28, 2018

Effects of lignan-rich whole grain rye vs whole grain wheat on short-chain fatty acids in human feces

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Increasing evidence suggest that short-chain fatty acids (SCFAs) play an important role for human health. SCFAs are speculated to reduce the risk of inflammatory diseases, obesity, type 2 diabetes, heart disease and other conditions (1). SCFAs are produced by the gut microbiota in the large bowel as a result of dietary fiber fermentation. SCFA derived from dietary fibre include acetate, propionate and butyrate and the latter is used as a primary energy source of colonocytes. One aim of this study was to evaluate the effects of lignan-rich whole grain rye (WGR) and lignan-low whole grain wheat (WGW) on the content of SCFA in fecal samples from men with metabolic syndrome as well as to correlate effects on cardiometabolic risk factors to SCFA. We conducted a randomized, 8 wk cross-over dietary intervention study with an 8 wk wash-out period. To further distinguish potential effects of lignans, lignan capsules were provided in WGR and placebo capsules were provided in WGW were daily supplied in rye intervention week 4-8. The SCFAs were quantified by gas chromatography coupled with triple-quadrupole mass spectrometry.
Lower content of butyrate, valeric acid and caproic acid were observed in WGW compared to WGR. Lignans appeared to have no effect on fecal SCFA concentrations. Furthermore, when comparing the content of fecal SCFAs before and after intervention in each dietary group, an increased content of acetate and propionate, and declined iso-valeric acid were seen after WGW intervention, but not in the WGR group. Our findings indicated that WGR and WGW have different impacts on the content of SCFA in feces, and we are currently investigating to what extent the differences are reflected by differences in gut microbiota and how SCFA correlate with cardiometabolic risk factors.

(1) Biskup I, et al. Plasma alkylresorcinols, biomarkers of whole-grain wheat and rye intake, and risk of type 2 diabetes in Scandinavian men and women. Am J Clin Nutr 2016. 104:88–96.

Title

The effect on P. aeruginosa secondary metabolome under antibiotic stress at sub-inhibitory concentrations

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Abstract text

It is well know that many antibiotics disturb bacterial cell even a low or sub-inhibitory concentrations. These effects have been studied mainly by means of genomics and transcriptomics approaches; however, antibiotic activity at the metabolic level is still not completely understood. Studying how antibiotics disturb the bacterial metabolism may bring a better understanding about the ability of bacteria to cope with antibiotic-induced stress responses.

In this project, we aim to understand how antibiotics may perturb bacterial metabolism of *P. aeruginosa*. For this, we make use of a metabolomics workflow to analyze the intracellular metabolome and to compare the response on the secondary metabolome under different antibiotic stress. As a first approach, we first look of the conditions necessary to observe a metabolic disturbance under antibiotic stress at both, inhibitory, sub-inhibitory and non-inhibitory concentrations. Interestingly, we found that even non-inhibitory concentrations of compound cause a strong metabolic dysregulation. Furthermore, we also corroborated that there is a specific response on the secondary metabolome according to the antibiotic class with which bacteria were treated.

Animals models for the identification of metabolic markers of environmental exposure

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Environmental research has been traditionally focused on biomonitoring of harmful chemicals in the environment. However, in the evaluation of the health impact of the environmental pollutants, screening of concentration levels of the pollutants only is not sufficient, because physiological responses to chemical exposure are highly individual. In recent years, use of metabolomics as a tool for the identification of early biomarkers sensitive to the effects of exposure has opened new opportunities in environmental exposure research.

Here we used two different animal models in order to study the impact of prenatal exposure to environmental chemicals on metabolism during the embryonic development. We used both an avian embryos model system and a fish embryo model to investigate the impact of prenatal perfluorooctane sulfonic acid (PFOS) and hexabromocyclododecane (HBCDD) exposure on lipid metabolism as determined by the lipidomics approach using UHPLC-QTOFMS.

Both PFOS and HBCDD exposures caused dose-dependent changes in lipidomic profiles in both model organisms. Particularly, after PFOS exposure, we observed upregulation of specific phospholipids associated with the phosphatidylethanolamine N-methyltransferase pathway, upregulation of triacylglycerols with low carbon number and double bond count as well as of lipotoxic ceramides and diacylglycerols. The HBCDD exposure also led to dose-dependent changes in the lipidomic profiles, particularly for triacylglycerols and specific phospholipids. Interestingly, we could identify these effect-based biomarkers also in ongoing human studies, thus indicating that the results from the animal models may be translatable to human studies.

Title
Hemolymph metabolites as monitored by NMR are tightly linked to cold tolerance of different Drosophila species

Authors (presenting author <u>underlined</u>)
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Abstract text

This is a study that shows that NMR is not necessary as insensitive as one might think and that once in a while one can get some overwhelmingly strong correlations between metabotype and phenotype:

Drosophila, like most insects, are susceptible to low temperatures, and will succumb to temperatures above the freezing point of their hemolymph. For these insects, cold exposure causes a loss of extracellular ion and water homeostasis, leading to chill injury and eventually death.

Here, we compared the hemolymph metabolite profiles of five Drosophila species with marked differences in chill tolerance. All species were examined under 'normal' thermal conditions (i.e. 20°C) and following cold exposure (4 h at 0°C).

Using NMR spectroscopy, we found that chill tolerance had a high correlation with the major variations in the hemolymph metabolome both at normal thermal conditions and following cold exposure.
The chill tolerant species have higher levels of sugars and free amino acids in their hemolymph, including classical 'cryoprotectants' such as trehalose and proline. In addition, chill tolerant species maintain a relatively stable hemolymph osmolality and metabolite profile when exposed to cold stress while sensitive species suffer from large increases in osmolality and massive changes in their metabolic profiles during a cold stress. We suggest that the larger contribution of classical cryoprotectants in chill-tolerant Drosophila plays a non-colligative role for cold tolerance that contributes to osmotic and ion homeostasis during cold exposure and, in addition, we discuss how these comparative differences may represent an evolutionary pathway toward more extreme cold tolerance of insects.

Title
Metabolomic profiling of potential biomarkers to associate environmental exposures with autoimmune diseases

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Abstract text

It is considered that majority of chronic diseases are caused not solely by genetic factors. Both external (environmental contaminants, radiation, climate, diet etc.) and internal (metabolism, gut microflora, ageing etc.) exposures play a large role in acquiring and developing diseases, including autoimmune disorders, such as type 1 diabetes mellitus. An improved understanding of how external and internal exposures interact during fetal growth and early life can expectantly help to explicate the causes of these complex pathologies.

In this project, a mother-child cohort related to an autoimmunity development was studied. Serum samples were extracted with HPLC-grade methanol. After centrifugation, supernatants were dried and derivatized prior to the analysis. A profiling of low-molecular weight and polar metabolites (amino acids, free fatty acids, sugar derivatives etc.) was performed in full-scan mode with a gas chromatograph coupled to a quadrupole time-of-flight mass spectrometer (GC-QTOFMS) to identify the discriminative elements of the metabolome.

The aim of the study is to define potential biomarkers during fetal and early life exposures that will further be used to find associations with autoimmune disease prevalence. Automated data processing tools will also be employed to distinguish signals between endogenous metabolites and environmental contaminants, as well as other exogenous chemicals, including not assigned to the MS libraries compounds.