LIPIDOMICS FORUM

13. - 15. November 2016

VENUE

ISAS e.V.
Otto-Hahn-Str. 6b
44227 Dortmund
Germany

ORGANIZERS

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AbstractBook

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Sunday | November 13

18:00 **Opening KEYNOTE**

Konrad Sandhoff

Membrane lipids regulate lysosomal sphingolipid catabolism, its enzymes and lipid transfer proteins

20:00 **DINNER**

Monday | November 14

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Prices for best Poster and Talks

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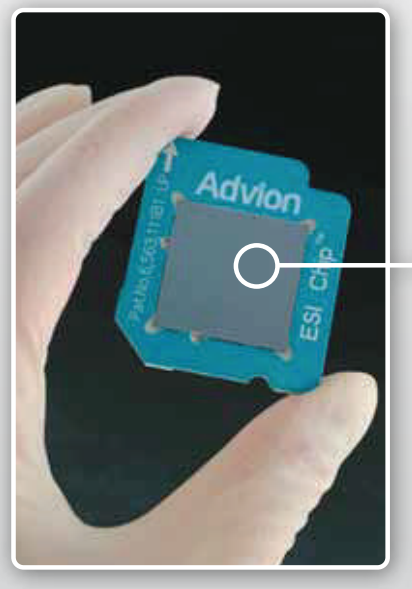

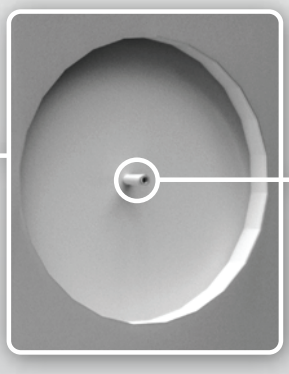

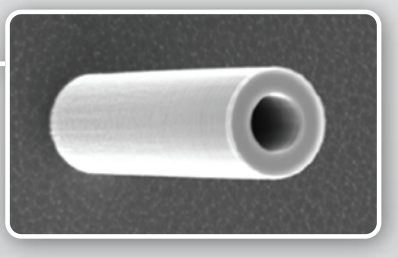

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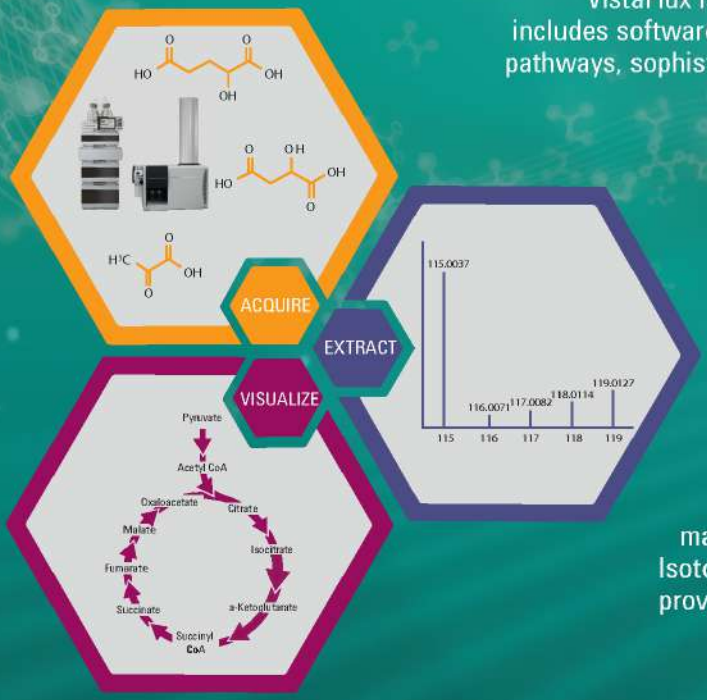

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Talks

The GOs and NO-GOs in lipidomics sample preparation

Dominik Schwudke[1]

[1]Department of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany

T 01

In this tutorial, I will discuss on basis of the classical extraction procedures of Folch and Bligh and Dyer what additional precautions have to be taken to conceptualize a fitting protocol for your lipidomics study. In this regard, importance is given to standardization for high-throughput and micro-scale procedures. I will share some of my experiences for optimizing everyday routine as well as how customized procedures could be implemented for analysing cell cultures, model organisms and clinical samples. In this regard, I look forward to a fruitful interaction about sampling, homogenization, extraction procedures and quality control measures in lipidomics sample preparation.

Natural variation of blood plasma lipids in healthy asian individuals

Markus Wenk[1]

[1]Department of Biochemistry, National University of Singapore, Singapore

T 02

Many unanswered questions remain as to the impact of genome natural variation in healthy populations. Biological variation can also be studied by assessing metabolic profiles within (within-subject variation) and across (between-subject variation) individuals. Numerous endogenous and exogenous factors contribute to biological variation and affect homeostatically controlled metabolic profiles. The influence of ethnicity remains a largely unknown contributor to between-subject variation. It is imperative to understand natural variations in basal lipid profiles across healthy individuals before we can study pathological conditions. This study aims to explore biological variation of glycerophospholipids, sphingolipids and sterols in human plasma from approximately 360 healthy Singaporeans, with an equal split across the major ethnic groups in Singapore (Chinese, Malay and Indian).

Essential role of the sphingolipid pathway in the maintenance of epidermal differentiation and whole body energy homeostasis

Fiona M. Watt[1], **Louise van der Weyden**[2]

[1]King's College London, London, United Kingdom
[2]Wellcome Trust Sanger Institute, Cambridge, United Kingdom

T 03 **PRICE**

The epidermis is the outermost layer of skin that acts as a barrier to protect the body from the external environment and control water and heat loss. This barrier function is established through the multistage differentiation of stem cells in the epidermis, and the presence of bioactive lipids such as ceramides, the levels of which are tightly regulated by a balance of ceramide synthase and ceramidase activities. Ceramides and their enzymes ceramidases are crucial components of the epithelial sphingolipid pathway. Here we reveal the essential role of alkaline ceramidase 1 (Acer1) in the skin, as Acer1-deficient (Acer-/-) mice showed elevated levels of different ceramide species in the skin, aberrant hair shaft cuticle formation and cyclic alopecia. We show that Acer1 is expressed specifically in differentiated interfollicular epidermis, *infundibulum* and *sebaceous* glands and consequently, Acer-/- mice showed significant alterations in their *infundibulum* and *sebaceous* gland architecture. Acer-/- skin also showed perturbed hair follicle stem cell compartments. These alterations in the epidermis led to Acer-/- mice showing increased transepidermal water loss and a hypermetabolism phenotype with associated reduction of fat content with age. Collectively, our study suggests that Acer1 is indispensible for mammalian skin and energy homeostasis and reveal new in vivo role of sphingolipid pathway.

[1] Kifayathullah Liakath-Ali, Valerie E. Vancollie, Christopher J. Lelliott, Anneliese O. Speak, David Lafont, Hayley J. Protheroe, Camilla Ingvorsen, Antonella Galli, Angela Green, Diane Gleeson, Ed Ryder, Leanne Glover, Gema Vizcay-Barrena, Natasha A. Karp, Mark J. Arends, Thomas Brenn, Sarah Spiegel, David J. Adams, Fiona M. Watt, and Louise van der Weyden. Alkaline ceramidase 1 is essential for mammalian skin homeostasis and regulating whole body energy expenditure. *Journal of Pathology*, 2016.

Accurate quantification of polyunsaturated glycerophospholipids by shotgun lipidomics

Kai Schuhmann[1], **HongKee Moon**[1], **Henrik Thomas** [1], **Andrej Shevchenko**[1]

[1]Max-Planck-Institut für molekulare Zellbiologie und Genetik, Dresden, Germany

T 04

Introduction

Carboxylate anions (CA) are employed for quantifying molecular species of glycerophospholipids (GPL) by MS^2. While CA of saturated fatty acids are stable during CID, CA of polyunsaturated fatty acids (PUFA) rapidly degrade and thus bias lipid quantification. We developed a method that circumvents CA instability and supports accurate quantification of PUFA containing GPL in shotgun lipidomics.

Method

We analyzed the MS^2 fragmentation of GPL on LTQ Velos and QExactive Orbitrap FTMS. Correlations of collision energy, fragment ion abundances, position and number of double bonds (DB) and length of the FA moieties were examined. Further, we developed a multidimensional mathematical model that enabled a simple and accurate way for correcting CA abundances.

Preliminary results

We observed that a larger number of DBs and their proximity to the carboxyl group drastically reduced the CA abundance during MS2 at elevated collision energy (CE). This introduces a major bias of more than 50% in quantification of PUFA-containing lipids compared to saturated lipids. While lower CE provide more equal response, it is impractical because of the sensitivity loss overall. Therefore, we developed a mathematical model for structural aspects of the FA moieties and CE and implemented this into a software to correct detected CA abundances. The model accuracy was validated using defined mixtures of synthetic lipids. Further, we applied our approach to quantify PUFA containing GPL in retina and brain total lipid extracts. Overall, abundance biases for physiologically important PUFA species could be reduced more than 4x and the deviation from expected values was below 20%.

In vivo synthesis of ^{13}C labeled reference lipids for absolute quantification by mass spectrometry

Evelyn Rampler[1]
Cristina Coman[2], Gerrit Hermann[3], Kristaps Klavins[4], Robert Ahrends[2], Gunda Koellensperger[1]

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[2]Leibniz-Institut für Analytische Wissenschaften - ISAS - e.V., Dortmund, Germany
[3]ISOtopic Solutions, Wien, Austria
[4]Biocrates Life Sciences AG, Innsbruck, Austria

T 05

Absolute quantification is an essential task in comprehensive lipidomics studies challenged by the high number of lipids, their chemical diversity, and their dynamic range (up to 7 orders of magnitude) of the lipidome. Currently, state of the art quantification comprises class specific internal standardization, i.e. applying one or two individually synthesized internal standards per lipid class. In this work, we propose a novel alternative calibration approach based on isotope dilution. For this purpose, a fast and efficient strategy for the production of ^{13}C labeled lipid library was introduced paving the way to comprehensive compound-specific internal standardization in mass spectrometry based assays. More specifically, the yeast *Pichia pastoris* served for in vivo synthesis of a ^{13}C labeled lipidome offering a good model for higher eukaryotes. More than 200 lipid species (from PA, PC, PE, PG, PI, PS, LysoGP, CL, DAG, TAG, DMPE, Cer, HexCer, IP, MIPC) were obtained from yeast extracts with an excellent ^{13}C labeling degree $> 99.5\%$, as determined by complementary high resolution mass spectrometry based shot-gun and LC-MS/MS analysis. In a first proof-of-principle study, different glycerophospholipids were quantified by the proposed isotope dilution strategy using reversed phase LC-MS. Applying this compound-specific quantification LOQs between 1 and 7 fmol absolute were observed and led to glycerophospholipid concentrations of 1 to 10 nmol per 108 starting yeast cell material. The analytical figures of merit were excellent with extraction recoveries of approximately 100% and an experimental repeatability lower than 10% for four individually prepared samples.

Selected applications of Orbitrap and FT-ICR mass spectrometry on lipid metabolism

Harald C. Köfeler[1]
Alexander Triebl[1], Martin Trötzmüller[1]

[1]Medical University of Graz, Graz, Austria

T 06

Lipidomics is driven by rapid advances in analytical technologies such as mass spectrometry and by getting insights at the level of lipid molecular species in understanding lipid metabolism and its dysregulation. The plethora of different lipids in biological systems requires the highest possible mass spectrometric resolution and mass accuracy, information about specific fragments by MS/MS, and chromatographic pre-separation, particularly for low abundant oxidized phospholipids. Our high resolution lipidomics platform depends on three selectivity criteria for separation and identification: retention time, exact mass at a resolution of 100 000, and collision induced dissociation (CID) fragment spectra in a linear ion trap. The versatility of this platform is highlighted by its application on metabolic fluxes in lung tumors.

Relevance of appropriate internal standards for accurate quantification using liquid chromatography tandem mass spectrometry - tauro-conjugated bile acids as an example

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Gerhard Liebisch[1], Christa Buechler[2]

[1]Institute of Clinical Chemistry and Laboratory Medicine, Regensburg University Hospital, Regensburg, Germany
[2]Department of Internal Medicine I, Regensburg University Hospital, Regensburg, Germany

T 07

Introduction: It is well established that matrix effects (MEs) may affect signal intensities in electrospray ionization (ESI) and thus may be considered as main challenge for quantitative LC-MS/MS. Stable isotope labeled (SIL) ISs are considered as gold standard because they closely resemble the properties of the analyte and show similar ionization efficiency and retention times. Frequently, SIL-ISs are either not available or very expensive and alternative ISs are used for quantification. Here, we present a systematic comparison of ISs from a routine LC-MS/MS method for bile acid (BA) analysis with a focus on tauro-conjugated BAs including stable isotope labeled (SIL) D5-tauro BAs.
Materials and Methods: BA analysis was performed with a previously developed and validated LC-MS/MS with minor modifications. Taurine conjugated BAs (TBAs) calibration curves were generated using different ISs. The 15 obtained internal standard curves were used for quantification of the respective TBA in quality controls (QCs) and patient samples. Quantification was performed in serum of up to 76 different.
Results: Both human serum based QCs and human serum samples were quantified with a variety of SIL-ISs. As expected, matching SIL-ISs provided the highest data quality (i.e. precision, accuracy). Both accuracy and precision of QCs and serum concentrations showed significant correlations. This provides evidence that calculation of matrix-based QC with various ISs could be applied for the selection of ISs whenever matching SILs are not available. Moreover, data calculated without ISs exhibited a poor data quality for both QCs and serum concentrations.
Conclusions: Here, we show that selection of ISs for quantification by LC-MS/MS is of enormous importance to achieve accurate and precise values in routine analysis of TBAs. Unsurprisingly, matching SILs show the best performance. Whenever SILs are not available, analyses of QCs based on sample matrix provide a tool to select ISs.

Novel lipid biomarkers for microbial ecology in complex environmental samples

Julius S. Lipp[1]
Lars P. Wörmer[1], Kevin W. Becker[2], Felix J. Elling[3], Xiaolei Liu[4], Travis B. Meador[1], Jan M. Schröder[1], Marcos Y. Yoshinaga[5], Chun Zhu[6], Kai-Uwe Hinrichs[1]

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[2]Dept. of Marine Chemistry and Geochemistry, Woods Hole Oceanographic Institution, Woods Hole, USA
[3]Dept. of Earth and Planetary Sciences, Harvard University, Cambridge, USA
[4]Dept. of Earth, Atmospheric and Planetary Sciences, Massachusetts Institute of Technology, Cambridge, USA
[5]Instituto de Química, Universidade de São Paulo, São Paulo, Brazil
[6]ExxonMobil Upstream Research Company, Spring, USA

T 08

Diversification of membrane lipids has been a crucial step in proliferation of microbial life, ecological expansion into new or changing habitats, and exploration of novel resources. This results in strong links between lipid composition and taxonomy, environmental conditions, and some particular metabolic activities, an association that is the base of the lipid biomarker concept. Applied to environmental and geological samples, lipid biomarkers can provide a wealth of information on geological and biological timescales, respectively.
We have developed a suite of complementary high-performance liquid chromatography mass spectrometry (HPLC-MS) analytical methods for the characterization of intact polar lipids and their apolar derivatives, quinones, and pigments with special emphasis on complex environmental samples. The protocols provide an unprecedented view of a rich lipid diversity from Archaea and Bacteria in environmental samples including microbial mats, water column, and shallow and deeply buried sediments with vastly differing environmental conditions. Among several newly discovered compounds were multiple glyceroldialkylglyceroltetraether (GDGT) lipids of archaeal and bacterial origin featuring structural modifications such as irregular branching patterns, additional unsaturation or hydroxylation, methyl- or ethyl-elongation of one glycerol backbone, or naturally-occurring degradation products [1-5].
Patterns of cyclization and alkyl chain length in the core lipid structures and polar head group-specific ratios of intact to core lipid concentrations distinguish microbial groups from different habitats and geochemical and environmental conditions. Our results facilitate the understanding of the role of microbial communities in past and present ecosystems.

[1] XL Liu, RE Summons, and KU Hinrichs. Extending the known range of glycerol ether lipids in the environment: structural assignments based on MS/MS fragmentation patterns. *Rap Commun Mass Spectrom*, 26:2295–2302, 2012.

[2] C Zhu, MY Yoshinaga, CA Peters, XL Liu, M Elvert, and KU Hinrichs. Identification and significance of unsaturated archaeal tetraether lipids in marine sediments. *Rap Commun Mass Spectrom*, 28:1144–1152, 2014.

[3] XL Liu, JS Lipp, JS Simpson, YS Lin, RE Summons, and KU Hinrichs. Mono- and dihydroxyl Glycerol Dibiphytanyl Glycerol Tetraethers in marine sediments: identification of both core and intact polar lipid forms. *Geochim Cosmochim Acta*, 89:102–115, 2012.

[4] C Zhu, TB Meador, W Dummann, and KU Hinrichs. Identification of unusual butanetriol dialkyl glycerol tetraether and pentanetriol dialkyl glycerol tetraether lipids in marine sediments. *Rap Commun Mass Spectrom*, 28:332–338, 2014.

[5] XL Liu, D Birgel, FJ Elling, PA Sutton, JS Lipp, R Zhu, C Zhang, M Könneke, J Peckmann, SJ Rowland, RE Summons, and KU Hinrichs. From ether to acid: A plausible degradation pathway of glycerol dialkyl glycerol tetraethers. *Geochim Cosmochim Acta*, 183:138–152, 2016.

Lipidomic characterization in different breast cancer tumor subtypes: a novel strategy for diagnostic detection

Mesut Bilgin[1]
Pavel Gromov[1], Marja Jäättelä[1], Irina Gromova[1]

[1]Department of Cell Death and Metabolism, Danish Cancer Society Research Center (DCRC), Copenhagen, Denmark

T 09

Breast cancer (BC) is one of the most common cancer types, accounting for 25% of all newly diagnosed cancers and 1.67 million patients worldwide in 2012. Based on the presence or absence of hormonal receptors (oestrogen and progestrogen) and human epidermal growth factor receptor 2, BC can be subdivided into four subtypes; luminal A, luminal B, triple negative/basal-like, and HER2, with distinct pathological behaviors and drug intervention.
The lipid metabolism plays important roles in cancer pathology and progression. Here, we profiled intracellular and secreted lipids for 23 different BC cell lines representing all four BC subtypes using an approach of quantitative shotgun lipidomics. Quantification of 25 lipid classes representing over 350 species illuminated lipid profiles, which were characteristic for the individual BC subtypes and clustered cell lines belonging to the same BC subtypes. For instance, all five BC cell lines representing the luminal A/B subtypes showed significantly higher PE levels intracellularly than those in the other subtypes at the expense of the PE O- levels. The luminal A/B subtypes showed high levels of PE species with single double bond (PE 32:1, 34:1, 36:1, and 38:1) or polyunsaturation (38:4, 38:5, 40:7, and 40:8) at the expense of the corresponding PE O- species. The data on secreted lipids showed quantitative profiles distinct from that of intracellular lipids. All together, the present study shows that the BC subtypes differ in their lipid profiles and suggests that lipid metabolism is differentially regulated among them with possible implication in cell physiology and pathology. This study illuminates the great potential of applied lipidomics within the cancer biology, drug intervention, and diagnostic detection.

Computer assisted identification and quantification of phospholipids using data dependent and independent LC-MS

Zhixu Ni[1,2]
Maria Fedorova[1,2]

[1]Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Leipzig, Germany
[2]Center for Biotechnology and Biomedicine, Universität Leipzig, Leipzig, Germany

T 10 **PRICE**

The alteration of the lipidome is an important part of a cellular response to various stress conditions. In the past decades, the non-targeted profiling by liquid chromatography coupled on-line to mass spectrometry (LC-MS) was used commonly to identify lipid species in different cell and tissue types. However, the non-targeted identification and quantification of phospholipids (PLs) in biological samples is often challenged by the extreme complexity of the sample matrix, the low abundance of vast number of PLs and the sophisticated data analysis workflows required to obtain reliable identification and precise quantification results.

Here we report a computer assisted high throughput data analysis workflow based on the combination of data-dependent (DDA) and data-independent (MS^E) acquisition techniques. A robust DDA method was used to acquire high quality MS/MS spectra for reliable identification of PL species, whereas MS^E data allowed to achieve more accurate quantification. Taking the advantages of both acquisition strategies, the overall quantification results gain higher confidence and robustness than individual workflows.

High-throughput automatic identification of PLs was performed by two in-house developed Python based tools. The mzML_Extractor was used to fetch all possible precursors with corresponding MS/MS scans. Data were transferred to mzML_Hunter to perform automatic assignment of MS/MS spectra and provide a ranked list of proposed structures based on an integrated scoring system. The outputs of mzML_Hunter contained a visual summary of each identified precursor including corresponding extracted ion chromatogram (XIC), MS full scan, isotope pattern distribution, and MS/MS spectrum with assigned product ion signals. This information was further imported into Progenesis QI (Nonlinear Dynamics, Newcastle, UK) and used for the quantification of PLs from corresponding MSE datasets.

By applying this workflow to the cardiomyocyte model of oxidative stress, we successfully identified 238 species from six main PL classes of which 118 lipids showed significant up/down regulation under oxidative stress.

Spatially-resolved lipidomics: Revealing localised lipid compositions and kinetics in tissue with high resolution mass spectrometry imaging

Shane R. Ellis[1]
Martin R. L. Paine[1], Mikhail E. Belov[2], Anthony D. Postle[3], Ron M. A. Heeren[1]

[1]The Maastricht Multimodal Molecular Imaging Institute, Maastricht University, Maastricht, Netherlands
[2]Spectroglyph, LLC, Kennewick, USA
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T 11

Conventional lipidomics relies on the extraction of lipids from biological material. However, this process results in the loss of all spatial information relating to the distributions of lipids in the sample. Given the heterogeneous nature of many biological tissues, including tumours, lipid localisation can provide valuable insight into localised biochemical processes and tissue state (i.e. disease diagnosis). Mass spectrometry imaging (MSI) is an extremely powerful approach to study the spatial distributions of lipids within tissues. This lecture will cover recent developments and applications both high-throughput MSI and high mass resolution technologies for studying localised lipid biochemistry. It will be shown how MSI and the revealed lipid distributions enable more accurate diagnosis of diseased tissues than conventional histology with examples from lung cancer xenografts and ischemic tissue studies. For example, the identification of cardiolipins hydrolysis products as markers for acute ischemia in kidney tissue and their relevance for organ transplant viability will be presented. In addition, we will present results relating to our development of a highly sensitivity ion-funnel based MALDI-MSI source and its application to lipid metabolism in respiratory diseases. Critically, we will demonstrate how such high mass resolutions MSI approaches allow localised lipid kinetics to be studied. In mouse models of respiratory disease we have infused D_9-choline prior to sacrifice and are able to monitor the uptake of labelled choline into PC lipid synthesis and thus spatially resolve areas of high and low lipid metabolism. These advances pave the way of dynamic MSI where lipid turnover rates are provided concurrently with imaging data thus providing a new window into tissue heterogeneity.

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Lipidomic biomarkers in vascular and metabolic diseases

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T 12

We performed morbidity (MB) and mortality (MT) studies in Diabesity patients quantifying lipid species by ESI-MS/MS in plasma, blood cells, and extracellular vesicles (EVs).
Analysis of lysophosphatidylcholine (LPC) revealed a decrease of most LPC species in Diabesity and negative correlations with C-reactive protein, body mass index (BMI), IL-6, and glycated hemoglobin levels. Correlating BMI ratio before and after $>10\%$ weight loss with the ratio of total LPC vs. individual LPC species showed significant negative relationships of LPC ratios with BMI ratios. Obesity was associated with decreased serum LPC levels, hypertension without obesity was associated with elevated LPC levels. Sphingosylphosphorylcholine (SPC) levels, together with soluble CD163 (Hb/Haptoglobin receptor) were significantly higher in Diabesity patients. Association of plasma lipid species with total MT and cardiovascular MT revealed protective effects of LPC species together with PUFA-phosphatidylcholine (PUFA-PC) species and long chain sphingomyelin (SM) and ceramide (Cer) species. In contrast, saturated and MUFA-PC species (e.g. PC32:0), and 16:0- and 24:1-containing SM and Cer species showed strongest positive association with MR. Among blood cells LPC16:0 and LPC18:0 predominate in red blood cells and platelets, and are low in monocytes, where LPC20:4, LPC22:5 and LPC22:6 predominate. LPC18:0 is high in granulocytes (PMN). Cer16:0 is high in PMN, while Cer20:0 and Cer22:0 predominate in platelets. Upon platelet senescence and activation LPC, lactosyl-Cer, sphingosine (SPH), sphinganine (SPA), and sphingosine-1-phosphate (S1P) are released with platelet EVs (PL-EVs), while lysophosphatidic acid (LPA) is formed in plasma. LPC, phosphatidic acid (PA) and SPH associate with plasma membrane derived PL-EVs. LPA, phosphatidylserine (PS), and Cer relate to exosomal PL-EVs. High-density lipoproteins (HDL) increase LPC and LPA in plasma and decrease S1P, hexosyl-Cer, and Cer. Therefore, lipid species levels in plasma, blood cells, and EVs may be valuable biomarkers for Diabesity care.

Shotgun lipidomics by high resolution mass spectrometry

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T 13

Lipids play essential roles in membrane structure and dynamics, energy homeostasis, and signal transduction. The lipidome of eukaryotic cells comprises several hundred to thousands of molecular lipid species produced by a metabolic network that interconnects and coordinates the metabolism of fatty acids, glycerophospholipids, glycerolipids, sphingolipids and sterol lipids. One of the grand challenges in cell physiology is to understand how cells regulate the activities of all lipid metabolic pathways simultaneously not only to maintain lipid homeostasis but also to remodel cellular processes and architecture. To delineate the regulatory landscape of lipid metabolism, we deploy systems biology approaches to reproducibly, comprehensively and quantitatively monitor both lipid molecules and the proteins that operate the lipid metabolic network. These approaches include high throughput lipidomics workflows capitalizing on nanoelectrospray ionization and high-resolution Orbitrap mass spectrometry combined with quantitative shotgun proteomics for time-resolved quantitative analysis of lipidome and proteome dynamics. The power of this platform is exemplified by our recent discoveries that: (i) activation of cardiolipin synthesis and remodeling supports mitochondrial biogenesis, (ii) down-regulation of de novo sterol synthesis machinery prompts differential turnover of lipid droplet-associated triacylglycerols and sterol esters, and (iii) sphingolipid metabolism is regulated in a previously unrecognized growth stage-specific manner. The application of this proteolipidomics technology serves as a new experimental paradigm for understanding, at unprecedented temporal resolution, how lipid metabolism is regulated and coordinated with the remodeling of cellular architecture and processes.

Mass spectrometry imaging of lipids – DOs and DON'Ts

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T 14

Mass spectrometry imaging (MS imaging) provides spatial and molecular information for a wide range of compounds. In contrast to most histochemical techniques, mass spectrometry imaging can differentiate modifications and does not require labelling of compounds. In this tutorial we will discuss critical steps and practical considerations in MS imaging with a special focus on lipids. This includes sample preparation, data acquisition and data processing/interpretation.

The last years have seen a number of collaborative efforts towards standardization and consolidation of MS imaging. One example is the common data format imzML which has become a de facto standard format for mass spectrometry imaging data. An important task for future MS imaging studies is the comparison and combination of methods and instrumentation across different laboratories. An approach on how to conduct such 'multicenter studies' for lipids will be discussed.

Contemporary mass spectrometric strategies for the saparation and identification of isomeric lipids

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T 15

Advances in mass spectrometry have been a significant driver in the emerging field of lipidomics. Improvements in speed, sensitivity and mass accuracy of modern instrumentation, although largely developed for other fields (e.g., proteomics and metabolomics), have improved our ability to detect lipids at very low concentrations within complex extracts. Peculiar challenges arise however, in the structural characterisation of lipids that are not directly addressed by generic performance enhancements in contemporary mass spectrometers. In particular, the differentiation of isomeric lipids including, double bond-positional isomers, sn-positional isomers and stereoisomers (e.g., *cis* and *trans* double bonds). It is often difficult or even impossible to distinguish such isomers by conventional mass spectrometric approaches in a manner that is also compatible with the high through-put workflows demanded in many applications. The inability to uniquely identify such important molecular features is a critical impediment to understanding the many and varied functions of lipids in biological systems. Our group is attempting to address these challenges through the development and application of mass spectrometric technologies specifically targeting the separation and identification of isomeric lipids. Application of these novel technologies in a range a biological contexts reveal a rich variety of isomeric lipids and thus provoke questions as to the true scope of molecular diversity in the lipidome.

Dexamethasone fails to inhibit the secretion of several inflammatory chemokines and counteracts the formation of both pro- and anti-inflammatory eicosanoids in human fibroblasts

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T 16

The aim of this study was to investigate the regulation of proteins, eicosanoids and metabolites in inflammatory stimulated and dexamethasone-treated human fibroblasts using untreated cells as controls. Proteome profiling demonstrated the strong induction of many chemokines and other effector proteins. For the analysis of eicosanoids, we successfully applied an in-house established data-dependent shotgun analysis method. Fourteen pro-inflammatory and nine anti-inflammatory eicosanoids were found induced upon inflammatory stimulation, while six others remained unaffected. Using targeted metabolomics, several acylcarnithins and sphingomyelins were found significantly downregulated. Treatment of inflammatory stimulated fibroblasts with the antiphlogistic drug dexamethasone abrogated the formation of both pro- and anti-inflammatory eicosanoids and restored normal levels of acylcarnithins but not of sphingomyelins. The inflammatory chemokines CXCL1, CXCL5, CXCL6, and complement C3, known to contribute to chronic inflammation, were not counter-regulated by dexamethasone in the stimulated fibroblasts in contrast to observations with leukocytes. Similar findings were obtained with human mesenchymal stem cells and results confirmed by targeted analysis with multiple reaction monitoring. Furthermore, proteome profiling demonstrated cell type-specificity regarding transcription factors regulating cytokine expression. The prevention of anti-inflammatory eicosanoid formation as well as the sustained secretion of chronic inflammation mediators in dexamethasone-treated fibroblasts may thus contribute to rebound inflammation.

Identification of unusual phospholipids in *Acanthamoeba castellanii*

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T 17

Acanthamoeba are opportunistic protozoan pathogens that may lead to sight-threatening keratitis and fatal granulomatous encephalitis. The successful prognosis requires early diagnosis and differentiation of pathogenic *Acanthamoeba* followed by an aggressive treatment regimen. The plasma membrane of *Acanthamoeba* consists of 25% phospholipids (PL). The presence of C20 and, recently reported, 28- and 30-carbon fatty acyl residues is characteristic of amoeba PL. A detailed knowledge about this unusual PL composition could help to differentiate Acanthamoeba from other parasites, e.g. bacteria and develop more efficient treatment strategies. Therefore, the detailed PL composition of *Acanthamoeba castellanii* was investigated in this study by ^{31}P nuclear magnetic resonance spectroscopy, thin-layer chromatography, gas chromatography, high performance liquid chromatography, and liquid chromatography-mass spectrometry. Normal and reversed phase liquid chromatography coupled with mass spectrometric detection was used for detailed characterization of the fatty acyl composition of each detected PL. The most abundant fatty acyl residues in each PL class were octadecanoyl (18:0), octadecenoyl (18:1 Δ9), and hexadecanoyl (16:0). However, some selected PLs contained also very long fatty acyl chains: the presence of 28- and 30-carbon fatty acyl residues was confirmed in phosphatidylethanolamine (PE), phosphatidylserine, phosphatidic acid and cardiolipin. The majority of these fatty acyl residues were also identified in PE that resulted in the following composition: 28:1/20:2, 30:2/18:1, 28:0/20:2, 30:2/20:4 and 30:3/20:3. The PL of amoebae are significantly different in comparison to other cells: for the first time, we describe unusual, very long chain fatty acids with Δ5-unsaturation (30:3 Δ5,21,24) and 30:2 Δ21,24 localized exclusively in specific phospholipid classes of *A. castellanii* protozoa that could serve as highly specific biomarkers for the presence of these microorganisms.

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Integrated software for data processing and analysis in direct infusion ultra-high resolution accurate mass spectrometry based "Top-Down" lipidomics workflows

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T 18

For direct infusion MS-based lipidomics workflows, ultra-high resolution accurate mass spectrometry (UHRAMS) coupled with selective derivatization of PE, PS and plasmalogen lipids provides a convenient solution to address isobaric and "sum-composition level" isomeric mass lipid overlap [1,2]. One remaining bottleneck limiting the wide-spread application of high-throughput untargeted lipidomics analysis is the lack of integrated software tools. Here, we describe the key features of LipidSearch 5.0 software designed specifically for top-down lipidomics analysis.

Global monophasic lipid extraction of polar and non-polar lipids, selective derivatization of aminophospholipids and plasmalogen ether-containing lipids, and UHRAMS analysis was performed using a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer. "Sum-composition level" lipid identification was performed by LipidSearch 5.0 software.

Key data processing features include mass re-calibration, Gaussian peak fitting of the monoisotopic peak, and Poisson modeling to identify and correct isotopic overlaps. Assignment of the monoisotopic peak is performed by searching against a user-defined database in SMILES format, enabling individual selection of lipid categories, class and subclass, total carbons and double bonds, positive or negative ion adducts, and definition of "fixed" and "variable" modifications. Isotopic peaks from the identified lipid are then removed and the process is repeated for all remaining peaks.

In less than 1 min at least 500-600 "sum-composition level" lipid species were confidently identified (after filtering) from crude lipid extracts. After normalization against internal standards, results are graphically overlaid for relative quantification, statistical comparison between groups, and exported for multivariate analysis. Positive and negative ion results are merged, providing higher confidence for lipid identification. MS/MS spectra are searched for the product ions predicted from "Sum composition level" ions identified during the MS level data processing. Unique and non-unique product ions are assessed and the MS/MS results are corrected for theoretical isotopic overlap.

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Comprehensive analysis of isoprenyl-phosphates via Q-TOF mass spectrometry

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T 19

Plants synthesize and provide essential metabolites for human health, as for example vitamin E. Vitamin E deficiency in humans results in severe neurological disorders, neuromuscular defects, and anemia. Vitamin E is synthesized via a complex biosynthetic pathway and comprises the tocopherols and tocotrienols.
Key intermediates in the biosynthesis of vitamin E and other isoprenoid-derived metabolites are isoprenyl-phosphates. Geranylgeranyl-diphosphate (GG-PP) is produced via the isoprenoid *de novo* synthesis from condensation of 4 isoprene units. Farnesyl-PP, another product from isoprenoid *de novo* synthesis, is required for the synthesis of sterols and steroid hormones.
During vitamin E synthesis in plants, homogentisate is prenylated using phytyl-diphosphate (phytyl-PP) or GG-PP. Phytyl-PP can be synthesized from GG-PP via reduction or by two subsequent phosphorylation steps from phytol released from chlorophyll (salvage pathway).
The levels of these isoprenyl-phosphates in *Arabidopsis* leaves and seeds have not been determined so far as tools were not available.
Understanding the complete biosynthetic pathway for vitamin E synthesis including the intermediates is of uttermost importance to benefit human health.
Therefore, we developed a method for the extraction and subsequent analysis of isoprenyl-phosphates via Q-TOF LC-MS/MS in as little as 10 mg of leaf or seed tissue. Furthermore, we synthesized internal standards for the absolute quantification of these compounds. This method provides novel insights into basic biosynthetic steps of isoprenoid-derived compounds in plants. We employed the LC-MS based analysis to quantify phytyl-P, phytyl-PP, GG-P, and GG-PP in vte1, hpt1, and vte5 Arabidopsis mutants with altered tocopherol contents to obtain comprehensive data on the metabolic changes in these mutants. Moreover, we used this method to study novel alternative routes for the synthesis of GG-PP, farnesyl-PP, and phytyl-PP.

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Adipose tissue sample analysis by high resolution NMR spectroscopy

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T 20

The interest in (phospho)lipids and their analysis is continuously increasing. Although the majority of analytical methods is based on mass spectrometry, nuclear magnetic resonance (NMR) spectroscopy (particularly ^{1}H, ^{13}C and ^{31}P) is also a useful method to investigate complex lipid samples. One of our major research interests is the investigation of adipose ("fat") tissue which is available in relatively huge amounts. Therefore, the poor sensitivity of NMR is not a major drawback.
We will show that high resolution NMR offers the following possibilities:
1) Using ^{1}H NMR spectroscopy the fatty acyl composition of triacylglycerols (TAG) and phospholipids can be determined in a single experiment and even with a higher accuracy in comparison to gas chromatography.
2) Using ^{13}C NMR the position of a fatty acyl residue in sn-1 or sn-2 position can be determined - although this works really accurately only if unsaturated residues are concerned.
3) Subsequent to derivatization with compounds such as 2-chloro-4,4,5,5-tetramethyldioxaphospholane even small amounts of mono- and diacylglycerols as well as other products of TAG degradation can be easily determined in complex TAG samples.
4) 31P NMR is a convenient method to differentiate virtually all phospholipid classes and enables (at least in selected cases) the determination of the position and the type of the fatty acyl residue. This method is also suitable to determine the activities of phospholipases and requires only a single standard.
In addition to these examples it will also be demonstrated that extraction of the samples is not absolutely necessary but the sample can be directly dissolved in suitable detergents. Therefore, potential extraction losses (particularly of highly polar phospholipids) can be minimized.

Elucidating a novel metabolic pathway of the neurotoxic 1-deoxysphingolipids

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T 21

Serine palmitoyltransferase (SPT) catalyzes the first step in the *de novo* synthesis of sphingolipids, typically condensing serine and palmitoyl-CoA. Under certain conditions SPT also uses alanine resulting in the cytotoxic 1-deoxysphingolipids (1-deoxySLs). Pathologically elevated 1-deoxySLs cause the inherited neuropathy HSAN1 and are also elevated in patients with metabolic syndrome and type 2 diabetes. Due to the missing C1-hydroxyl group, 1-deoxySLs cannot be degraded by the canonical pathways and therefore they are widely assumed to be "dead-end" metabolites.
We used a variety of metabolic labeling approaches in combination with high resolution high accuracy mass spectrometry and metabolic profiling workflows to identify novel downstream 1-deoxySL metabolites. The formation of these novel 1-deoxySL metabolites was modulated using specific chemical inhibitors and inducers, as well gene overexpression.
In this manner we identified 8 novel 1-deoxySL metabolites which were singly or doubly hydroxylated, or further desaturated, forming 3 branches of a downstream metabolic pathway. We elucidated the order of the metabolic pathway by treating cells with each of the individually purified metabolites. Furthermore, we found that inhibition or induction of the CYP4F enzyme subfamily prevented or increased the formation of these downstream metabolites, respectively.
While cytotoxic 1-deoxySLs are not metabolized by the canonical pathways, we showed for the first time that they are in fact further metabolized by CYP4F enzymes to 8 novel downstream metabolites. A number of reports have suggested that this enzyme subfamily is downregulated in mouse models of obesity and fatty liver disease. Therefore, this metabolic pathway may be exploited in the future as a novel therapeutic target to reduce 1-deoxySL levels in metabolic syndrome and type 2 diabetes patients.

Healthy plasma lipidome

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T 22

Lipidomics of human blood plasma is an emerging biomarker discovery approach that compares lipid profiles under pathological and physiologically normal conditions. While lipidome changes associated with different manifestations of metabolic syndrome (e.g. obesity, hypertension and insulin resistance), type 1 and type 2 diabetes or cardiovascular diseases, to mention only a few most common pathologies, were revealed in numerous epidemiological studies, basal properties of the plasma lipidome received surprisingly little attention. Lipid metabolism differs between genders, but if and how is it reflected by plasma lipids? Are they affected by the levels of sex hormones and do they change during the menstruation cycle? Do male and female lipidomes react differently on the dietary challenges?

To address these and other basic questions, we assembled a cohort of 71 Caucasians under the age of 33 years. Each individual had clean medical record with no factors comorbid to common metabolic disorders and 35 clinical blood test and anthropometric indices were within the medical norm. Molar concentrations of 281 molecular species from 27 major lipid classes were quantified by shotgun profiling and LC-MS/MS.

We established that gender is a major and previously underestimated lipidomic factor that affects 112 out of the total of 281 quantified plasma lipids and the total abundance of 21 out of 27 lipid classes even [1]. Mean values of anthropometric (BMI, WHR) and clinical (blood pressure, LDL, HDL) indices are not covariate to gender discriminative lipids. Gender impact is strongly enhanced by hormonal contraceptives, mostly affecting the concentration of glycerophospholipids and glycerolipids. Concentrations of sphingomyelins and glycosphingolipids were significantly different between the genders, but in contrast to glycerophospholipids, were not affected by contraceptives. Interestingly, we observed no strong correlation of the levels of sex hormones (estradiol, testosterone) and plasma lipids. Within gender-restricted cohorts lipidomics revealed a compositional signature corroborating clinical indices trends towards developing a metabolic syndrome. We propose that the healthy plasma lipidome is a valuable resource for biomarker discovery that helps to recognize early, relatively minor, yet highly specific signs of emerging metabolic abnormalities, which later could be obscured by massive changes associated with unfolding of the metabolic syndrome.

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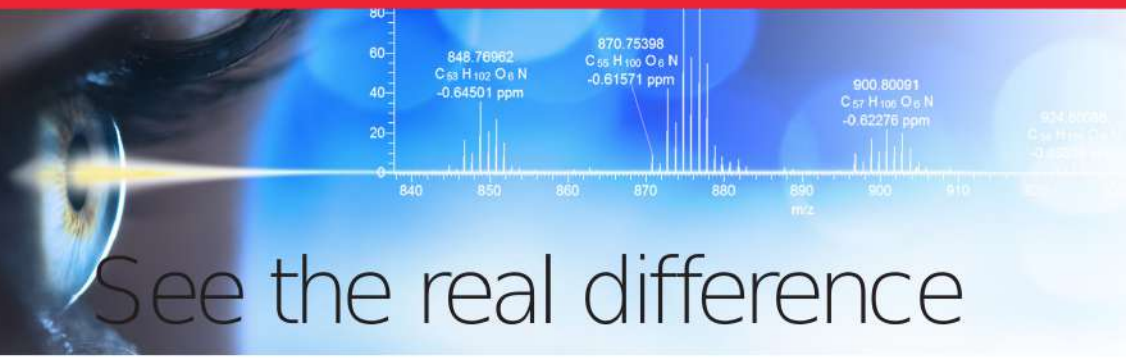

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Posters

Maintenance of sphingolipid homeostasis: A crucial role for endosomal vesicular recycling

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P 01

Sphingolipids and their metabolites are essential structural components of membranes and act as signaling molecules. Maintaining sphingolipid homeostasis is crucial to sustain membrane integrity and trafficking. Accumulation of sphingolipid metabolites can cause severe neurodegenerative disorders.
Capitalizing on unbiased genetic, proteomic and lipidomics approaches, we have discovered an essential role for endolysosomal trafficking in maintaining sphingolipid homeostasis. While most research on the Golgi-Associated Retrograde Protein (GARP) and other trafficking complexes focuses on protein distribution, we found that this complex has an essential role in maintaining sphingolipid distribution and homeostasis. Mutations in the GARP complex cause the neurodegenerative disorder progressive cerebello-cerebral atrophy type 2 (PCCA2) and lead to multiple defects in cells including problems in lysosomal function, sterol distribution and ion homeostasis. Importantly, most of these defects can be improved by chemical inhibition of sphingolipid biosynthesis in yeast as well as in human cells suggesting that sphingolipid metabolite accumulation is causative. In addition, sphingolipid biosynthesis inhibition has beneficial effects in a mouse model of neurodegeneration caused by a mutation in the GARP complex subunit Vps54. Together, our data suggest that sphingolipid Metabolite accumulation may be a feature contributing to PCCA2 and possibly other neurological disorders with connections to endo/lysosomal trafficking.

Monitoring the kinetics of phospholipase A2-digestion of oxidized phospholipids by MALDI-TOF MS

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P 02 PRICE

The enzyme phospholipase A2 (PLA2) plays very important physiological roles. It digests phospholipids (PL) by hydrolyzing the fatty acyl residue at the sn-2 position generating both lysophospholipids (LPL) and free fatty acids (FFA). During inflammatory diseases, reactive oxygen species (such as HOCl) lead to the formation of oxidatively modified PL (e.g., chlorohydrins). Upon PLA2 digestion, oxidized PL lead to the release of oxidized FFA, which may be converted into messenger molecules (leukotrienes or thromboxanes). Due to an excess of oxidized PL favoring inflammatory processes, and thus the development of chronic inflammatory diseases, the oxidation of PL has to be regulated tightly.

It is still widely unknown to which extent oxidized PL (including the impact of the position of the oxidized unsaturated fatty acyl residue (*sn*-1 vs *sn*-2) and different headgroups) are digested by PLA2.

Normally, the determination of the PLA2 activity is based on the detection either of the released amount of LPL or the FFA subsequent to PLA2 digestion of a known PL substrate. Commercially available kits often use PL with fluorescence or radioactive labels to improve the sensitivity.

MALDI-TOF MS is a suitable method to analyze PL and offers some important advantages: (i) the interpretation of MALDI mass spectra is very simple, because singly charged ions are nearly exclusively generated, (ii) MALDI mass spectra can be very easily and rapidly acquired, and (iii) MALDI MS tolerates salts and contaminations.

The aim of this study is the investigation of the (oxidized) PL digestibility by PLA2 using MALDI-TOF MS to elucidate the PL/ LPL ratios. We show that oxidative modifications of PLs (e.g. by HOCl) have a considerable impact on the PLA2 digestibility and, moreover, MALDI MS is a convenient and reliable tool to investigate the related changes even without internal standards.

A convenient high resolution NMR approach to identify the positions of acyl residues in triacylglycerols

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P 03 PRICE

Triacylglycerols (TAG) are major constituents of vegetable oils and fatty tissues. Detailed TAG analysis (regarding the fatty acyl composition and the positions of these residues at the glycerol) is even nowadays challenging, because the presence of three (often different) fatty acyl residues leads to many different, isomeric species. While the overall fatty acyl composition of TAG can be determined by gas chromatography/mass spectrometry (GC/MS), all the positional information is lost completely by this approach, because saponification of the sample is required. Thus, GC/MS is often replaced or combined with LC/MS to provide information about the fatty acyl residues in a given TAG. However, a precise differentiation between various isomers remains difficult. Here, we show that high resolution ^{13}C NMR spectroscopy is an excellent method to determine the position of the related fatty acyl residues in a given TAG molecule. The chemical shift of the esterified fatty acids depends on their position within the TAG molecule. With the help of ^{13}C NMR, fatty acyl residues in sn-1 and sn-2 position can be differentiated and all possible TAG isomers ranked according to their probability of occurrence. The NMR data were combined with MALDI MS data in order to calculate the complete TAG composition. Although the ^{13}C NMR method is particularly applicable to unsaturated TAG, this approach can be regarded as a significant methodological progress in this field.

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Lipidomic strategies for the analysis of fat metabolism dysfunction in the model organism *C. elegans*.

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P 04 PRICE

C. elegans is considered a valid model organism for studying the ageing process due to its short life span and easy genetic manipulation. In recent years, *C. elegans* was shown to become increasingly popular as a model for investigating lipid metabolism and lipid accumulation.
Although many genes playing a role in the fat metabolism have been studied, the lipid composition and lipidome change in nematodes mutants with lipid dysfunction has been investigated poorly.
Here, we used a mass spectrometry approach to study the lipidome of *C. elegans* mutants with abnormal fat accumulation. In this study, we focused on three specific mutations, maoc-1, the orthologue of the human 17-β-hydroxysteroid dehydrogenase IV, which is involved in the first steps of the peroxisomal beta oxidation and daf-22, which corresponds to human orthologues of the sterol carrier proteins (SCP2) and catalyzes the final step of the peroxisomal beta oxidation.
The lipid fraction was analyzed by shotgun lipidomics using a high resolution mass spectrometry (HRMS). Lipid identification was achieved using the open-source software LipidXplorer. Previous to semi-quantitative analysis, the lipid extract was subjected to RP-LC separation and analyzed using the features of peak detection, peak picking, and peak alignment present in the software Compound discoverer. Likewise, methyl esters of fatty acids were obtained by silylation reaction and analyzed by GC-QQQ.
For the first time, we provide a deep coverage of the lipidome of maoc-1 and daf-22. This analysis reveals that genomic perturbation of genes involved in the peroxisomal beta oxidation results in a change in the lipid composition.
This broad lipid characterization will help to understand the role of the peroxisomal beta oxidation in fat metabolism and opens new questions on the effect and the role of the different lipid compositions may have on cellular metabolism.

CsCl addition reduces ion suppression effects in the MALDI mass spectra of triacylglycerol and phosphatidylcholine mixtures and adipose tissue extracts

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Ion suppression is a known disadvantage in mixture analysis. MALDI mass spectra of crude adipose tissue extracts are dominated by triacylglycerol (TAG) signals while less abundant phospholipids such as phosphatidylcholines (PC) and particularly phosphatidylethanolamines (PE) are suppressed. We have found that the saturation of the matrix solution with solid CsCl helps to overcome this problem and this approach has the particular advantage of simple performance. Another advantage of the addition of Cs^+ ions is the ability to detect differences in the fatty acyl compositions of phospholipids, because interferences between different adducts can be minimized.

Selected lipid mixtures of known compositions and organic adipose tissue extracts were investigated by positive ion MALDI-TOF MS. 2,5-dihydroxybenzoic acid (DHB) in methanol was used as matrix. In selected cases the methanolic DHB solution was saturated by the addition of different solid salts (NaCl, KCl, RbCl and CsCl). Studies on the solubilities of the salts in methanol and the interaction with DHB were also performed. Saturation of the DHB matrix with solid CsCl leads to tremendous intensity differences, i.e. the intensities of the TAG signals (which otherwise dominate the mass spectra) are reduced significantly. In contrast, the intensity of small signals of phospholipids increases considerably. Decrease of the TAG signals intensity is caused by (a) the increased fragmentation of the corresponding alkali metal adducts and (b) the considerable size of the Cs^+ ion which prevents successful analyte ionization.

This study shows that the addition of CsCl is a convenient method to overcome ion suppression in samples with an excess of TAGs and improves the detectability of otherwise invisible or weak (PE and PC) phospholipid ions. This analysis can be performed rapidly and without the need of previous separation of the sample into the individual lipid classes, is time-saving, and provides reliable data in a single measurement.

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The extraordinary development of kangaroo sperm

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The genital tract of metatheria is characterized by many anatomical and physiological differences compared to eutherian species. For example, male kangaroos lack seminal vesicles, coagulating glands and ampullae, whereas the prostate is enlarged and bulbo-urethral glands are well established. After ejaculation a subsequent coagulation of the ejaculate occurs. Kangaroo sperm show dramatic morphological changes during their transit through the epididymis and their subsequent maturation. The outer appearance of the gametes changes from a T-shaped head tail orientation to a streamlined form. Even though there are some hypotheses, structural mechanisms underlying these adaptions are not yet well understood.
Cryopreservation of kangaroo sperm has not yet been successful, and no promising cryopreservation protocol has been established so far. However, the conservation of gametes is extremely important especially regarding the preservation of endangered species. The composition of the plasma membrane might be responsible for difficulties in cryopreservation. Lipids, as the main components, affect the physical properties of biological membranes and play a major role in sperm maturation. Therefore, the knowledge of the lipid composition is crucial for any further step regarding the preservation of the species.
To elucidate the tremendous developmental changes of these extraordinary sperm cells the lipid composition of the kangaroo caput and cauda sperm membrane was investigated by MALDI-TOF and ESI-IT as well as tandem mass spectrometry and NMR analyses. PC 16:0/20:3 and PC 18:0alkyl/22:3 could be detected as the main constituents of caput and cauda sperm, respectively. Eicosatrienoic and docosatrienoic acid contain an uneven number of double bonds and have to these authors best knowledge never been detected in male gametes of any species before.

Towards quantitative lipid imaging: Comparative studies on mouse brain using high-resolution MALDI-MS imaging followed by nano-HPLC-ESI mass spectrometry of laser micro-dissected brain regions

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MALDI-MSI delivers valuable information about the localization of endogenous biomolecules (e.g., phospho- and glycolipids) in tissue sections. Because varying extraction efficiencies and ion suppression effects the obtained ion images and do not generally allow an assessment about the absolute abundance of a particular compound of interest. To obtain this important quantitative information, most typical internal standards are added. Here, we used a combination of laser-micro-dissection and nano-HPLC-ESI-MS to determine the lipid content in different regions of mouse brain, which were analyzed by UV-MALDI-MSI beforehand. The overall goal was to obtain an improved understanding about the factors affecting the ion yields in MALDI-MSI of phospho- and glycolipids in dependence of the biological matrix.
16 µm-thick mouse cerebellum sections were thaw-mounted on laser-micro-dissection slides and coated with 2,5 dihydroxybencoic acid matrix. MS-images were obtained with a Synapt G2-S mass spectrometer (Waters) using a laser spot size of 10 µm in diameter and a pitch size of 50 µm. In this way, the largest fraction of the tissue sections was not interrogated by the MALDI laser. The MSI analysis regions of interest were identified and these areas micro-dissected using a PALM Microbeam system (Zeiss). Lipids were extracted using methanol/MTBE (1:3, v/v) and separated with a nano-HPLC system (Ultimate 3000, Thermo Fisher Scientific) using a C18 column. The HPLC system was coupled to the nano-ESI source of a QToF-I mass spectrometer (Micromass).
Chromatography allowed the separation and identification of several lipid classes. Circumventing possible ion suppression effects using the nano-HPLC separation enabled to characterize the lipid content of the different regions of murine cerebellum independent of its biological matrix. Direct comparison with MALDI-MSI data allowed characterizing possible interdependence of lipid species and suppression effects. Greater knowledge of ionization efficiencies and ion suppression may in the future enable dedicated untargeted quantification strategies in MALDI-MSI of lipids.

Elucidation of double bond positions in lipids by means of ozonolysis followed by ultraviolet laser desorption ionization mass spectrometry

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In many physiological processes, the biological activity of lipids is critically determined by the position of the C=C bond in unsaturated carbohydrate chains. One powerful analytical tool to unravel this stereochemical information is via ozonolysis. This method is used both off-line and on-line [1] and results in the formation of two sets of characteristic fragments that unambiguously identify the double bond position. Here we used off-line ozonolysis in combination with UV-laser desorption ionization mass spectrometry (UV-LDI-MS). We used a modified MALDI QStar mass spectrometer (AB Sciex) and used etched silver foils as substrates; UV-LDI-MS with these foils gives rise to molecular $[M + Ag]^+$ ions for numerous classes of lipids and LODs in the low picomole range [2]. We tested our method for a range of different lipid classes including straight chain hydrocarbons, free fatty acids, phosphor- and glycosphingolipids, as well as triacylglycerols. As a complex biological sample, a blend of cuticular lipid extracts from *Drosophila melanogaster* was moreover analyzed. In most cases, the lipids could be analyzed both as intact molecular species and, after ozonolysis directly on the substrate, as the expected characteristic fragments. Next to showing selected examples we present a time course study that revealed variable reaction rates for different substances. For example, the alkene 9Z-C23:1 reacted faster with O_3 than the singly unsaturated free fatty acid 9Z-18:1. Even under the relatively low O_3 concentration conditions as produced by a simple ozone generator (ACT-300, REDOzone) in a home-built chamber, in most cases a full conversion of the C=C bonds to ozonides and fragments was obtained within 120 s.

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Profiling of constitutional isomeric phospholipids by hydrophilic interaction LC-ESI-MSMS

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Phospholipids (PL) are important lipids, which inherit multiple functions in biological systems. These lipids are divided into classes regarding their polar head group which is linked to the glycerol backbone or to the glycerolphosphate backbone. Important representatives of phospholipids are phosphatidylcholines (PC), phosphatidylethanolamines (PE), phosphatidylserines (PS), phosphatidylglycerols (PG), phosphatidylinositols (PI), and bis(monoacylglycero)phosphates (BMP).

Especially, BMP is an interesting PL. BMP is a configurational isomer of PG, which is why it has been erroneously determined as PG for a long time. Only in 1967, BODY AND GRAY discovered BMP in pig and rabbit lungs.[1] BMP was found in the most mammalian tissues with a share of 1 - 18% of the total phospholipids.[2] Several reasons render BMP to an interesting lipid: Its biochemical properties (unusual selectivity and binding of fatty acids), its relevance for multiple biological activities (including cholesterol homeostasis), its specific cellular localization, and its important role in various diseases (including lysosomal disorders or breast cancer).[2][3] Several studies indicate that BMP can be used as biological marker for various diseases, because diseased tissue is often characterized by higher contents of BMP.

Also, important structural information can be obtained by MS^n. However, the constitutional isomers PC and BMP show highly similar MS/MS spectra. Therefore, differentiation of these lipids solely based on MS is not possible and hence chromatographic separation is mandatory. Consequently, we developed an HPLC method based on hydrophilic interaction liquid chromatography (HILIC) which provides a good opportunity to separate the polar lipid classes.

The applicability of the developed phospholipid-profiling method based on HILIC-ESI-MS/MS is demonstrated by analysis of polar lipids from MCF7 breast cancer cells.

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A HILIC-based Liquid Chromatography coupled tandem mass spectrometry approach for the separation of the glycosphingolipid isoforms glucosylceramide and galactosylceramide in kidney tissue

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P 10 **PRICE**

Cell-type specifical mammals synthesize the stereoisomeric hexosylceramides β-galactosylceramide and β-glucosylceramide, which are involved in several diseases such as diabetes, polycystic kidney disease, or cancer. Bacteroides, however, can produce α-galactosylceramide, which is the most potent stimulus for iNKT cells. To dissect the contribution of the individual stereoisomers to pathologies, we established a HILIC-based LC-MS/MS method with propionitrile using mouse kidney lipid extracts.

Using propionitrile instead of acetonitrile in solvent A of the liquid chromatography (LC) gradient we improved the resolution of the Waters Cortecs HILIC (150×2.1) column and could separate β-glucosylceramide from β-galactosylceramide with a resolution of $R > 2$. Additionally, we could separate the anomeric α- and β-galactosylceramides with a resolution of $R > 1.5$. Analysing raw lipid extracts of a mouse renal cortex, we could distinguish and quantify 14 different β-glucosylceramide and 15 different β-galactosylceramide species with this method.

Endogenous β-GlcCer, β-GalCer, and α-GalCer isomers can be separated, identified (including the ceramide anchor composition), and quantified by using a Waters Cortecs HILIC UPLC column and a propionitril/methanol LC gradient without additional derivation steps.

Localization of double bonds in complex lipids

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Lipids are a group of biomolecules with a broad variety of chemical structures, makeing them suitable for various tasks in a number of different organisms. Double bonds have an influence on lipid's chemical, biochemical, and biophysical properties. Because the localization of double bonds in lipids is still a difficult task regarding their structural diversity and possible complexities of available mixtures, the development of a new method addressing those challenges is required. The photochemical Paternò Büchi (PB) reaction based on the binding of acetone to the double bonds was used. Precise localization was achieved by tandem MS and diagnostic fragments [1].

After irradiation of UV light (254 nm), a molecule with a mass increase of 58 Da was detected in addition to the deprotonated oleic acid. The acetone adduct was formed due to the reaction of acetone with the double bond resulting in an oxetane. MS/MS experiments generated diagnostic fragments allowing the localization of the double bond position. Based on the experiments with oleic acid, the online reaction was carried out with lipids having a more complex structure. Starting with monoolein, a glycerol ester of oleic acid, comparable results to the previous experiments of the free fatty acid were achieved. Acetone adducts were formed, which were fragmented first to the derivatized free oleic acid and subsequently to their diagnostic fragments. A successful coupling of HPLC with the online reaction was demonstrated by using standards of a bis(monoacylglycero)phosphate and a phosphatidylglycerol. The phospholipids are constitutional isomers whose analytic and successful localization of its double bond positions requires a HPLC separation. Based on an achieved separation of a mixture, containing two of the phospholipids, the online reaction and pinpointing of existing double bonds was performed analogously to the experiments with monoolein.

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Discovering potential diabetic lipid biomarkers using HRAM LC-MS-MS approach on a hybrid quadrupole – high field orbitrap mass spectrometer

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Lipids play a key role in cell, tissue and organ physiology with diseases such as diabetes which involve disruption of their metabolic enzymes and pathways. Identification of unique lipid biomarkers to distinguish healthy humans compared to those with a disease can have an impact on the early detection of diseases and personalized medicine. Here we demonstrate that HRAM LC MS-MS approach on a hybrid quadrupole high field Orbitrap mass spectrometer enables rapid putative biomarker discovery through lipidomics profiling experiments. In these experiments phenotypic ZDF rat plasma (fatty vs. lean wild type) were used to demonstrate the capabilities of this method.

Cardiolipin measurement by liquid-chromatography-mass spectrometry in plants

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Cardiolipin (CL) is an anionic lipid exclusively found in bacteria and in the inner mitochondrial membrane of eukaryotes. Because of its low abundance, CL measurements by mass spectrometry are notoriously difficult. A number of protocols for the determination of CL content and molecular species composition from bacteria and animals were established. However, the molecular species composition of CL from plants has never been determined, given that because of the high abundance of chloroplast membranes in a plant leaf cell, the amount of CL is even lower as compared to animal cells. Therefore, the measurement of CL is hampered by the presence of other interfering lipids, especially galactolipids and neutral lipids. Here, we present a new method for analyzing CL from plants. To reduce the amount of interfering substances, we separated CL and other acidic lipids from the non-acidic lipids via anion exchange chromatography using solid phase extraction on a DEAE cellulose column. A Quadrupole Time-of-Flight (Q-ToF) LC/MS instrument was used in the negative mode for determination of CL content and molecular species composition. The separation of molecular species of CL and other acidic lipids (mainly PI, PG, and SQDG) was performed on a reverse phase column. Lipid extracts of different plant species, including *Arabidopsis*, mung bean, spinach, barley and tobacco and different plant tissues (leaves, hypocotyl, roots) were analyzed, and up to 23 different molecular species of CL could be detected [1]. The molecular species distribution among the plants was very similar and dominated by 72:X species with highly unsaturated acyl groups, mainly linoleic and linolenic acid. The establishment of this method for quantification of CL in plants provides the means to further investigate the mechanism of CL remodeling during stress for a better understanding of the role of CL and its metabolism in plants.

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Picosecond infrared laser desorption of lipids from porcine brain areas

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Lipids are essential for the general functioning of an organism. They serve as biomarkers for diseases such as cancer, HIV, and metabolic dysfunctions like cystic fibrosis.
Desorption by impulsive vibrational excitation (DIVE) is a homogenisation method of the picoseconds infrared laser (PIRL). It is an ultrafast and soft homogenization technique, which allows for the gentle extraction of biomolecules such as lipids from tissues or cells.
The goal of this study was to investigate the adequacy of PIRL for the extraction of lipids and the possible distinction of porcine brain areas.
The cerebral cortex of a domestic pig was either a) ablated and the lipids extracted before MS or b) ablated and measured directly with MS or c) classically homogenised using a mortar, pestle, and a dounce tissue grinder, followed by MS analysis. Both positive and negative nanoESI-MS/MS with data dependent acquisition (DDA) was employed and the resulting data processed.
More than 100 lipids were identified from all methods with the majority being glycerophospholipids. Principal component analysis (PCA) of the m/z signal intensity profiles from the full-scan-spectra showed approx. 98.54% similarity between the three methods.
The distinguished brain areas with a PCA in negative ion mode included; cerebral cortex, hypophysis, cerebellum, and mesencephalon. This was possible for the PIRL-ablated samples, which were measured directly without extraction. Other areas such as the thalamus, pallium cerebri, corpus callosum, and basal ganglia could not be separated.
The possibility of PIRL-DIVE extraction of lipids from brain tissue was confirmed, MS analysis without further lipid extraction was established, and several brain areas could be differentiated.

Brown and beige adipocyte lipoprotein lipase activity controls metabolic flux through the HDL compartment

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P 15 **PRICE**

Brown Adipose Tissue (BAT) activation is a promising approach to treat obesity. BAT activation lowers blood lipids by accelerating plasma triglyceride clearance into BAT. However, not only the amount of lipids but rather the complex composition of lipids controls cellular metabolic homeostasis and potential organ-specific lipotoxic effects. Here, we investigate the impact of BAT activation on circulating lipoproteins and systemic HDL-mediated cholesterol metabolism in mice using non-targeted lipidomic approaches.
BAT was activated in wild-type mice, apolipoprotein AV-knockout mice (ApoAV-KO), or mice with adipocyte specific deletion of lipoprotein lipase (aLKO) by cold exposure (6°C) or injection of the selective beta3-agonist CL316,243. Plasma lipoprotein profiling was performed using FPLC. Lipoprotein fractions were extracted using the method of Folch. Lipidomic analysis were conducted on a Dionex3000 UPLC (Column: Kinetex C18, 150x2.1 mm; 1.7 µm (Phenomenex)) coupled to an ESI-UHR-Q-TOF (maXis, Bruker Daltonik). HDL function was assessed *in vivo* using a reverse cholesterol transport assay or by measuring the hepatic uptake and plasma clearance of radioactive-labeled HDL.
BAT activation resulted in elevated HDL cholesterol levels in hyperlipidemic ApoAV-KO mice. A significant increase in cholesterol macrophage-to-feces efflux rate indicates improved HDL function after BAT activation. In accordance with this, we observed remodeling of main lipid classes in circulating HDL, which may influence HDL properties and function. Notably, in aIKO-mice not only the remodeling of the triglyceride moiety of HDL was blunted, but also the hepatic uptake of HDL-derived cholesterol was decreased indicating a functional role of the lipoprotein lipase (LPL) activity in HDL-mediated cholesterol metabolism.
In conclusion, BAT activation accelerates reverse cholesterol transport. Mechanistically, we show that lipolysis by adipocyte LPL in the blood stream drives HDL remodeling and turnover. Our findings confirm the concept that systemic metabolic flux regulated by the high metabolic activity of thermogenic adipocytes determines the atheroprotective properties of HDL.

Cold regulated bile acid synthesis shapes the gut microbiome

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Brown Adipose Tissue (BAT) activation increases energy expenditure and stimulates food intake. While some nutrients are metabolized directly by BAT, increased intake of cholesterol may be harmful and therefore requires alternative metabolic routes. Here, we evaluate the effects of BAT activation on systemic cholesterol and bile acid (BA) metabolism.

Mice were fed a Westerntype diet (0.2% cholesterol) and housed at 28°C or 4°C for 7 days to activate BAT. For quantitative LC-ESI-MS/MS-based analysis, bile acids and hydroxy sterols were extracted from tissues and biofluids via a simple methanol liquid-liquid extraction and spiked with internal standards. Measurements of extracts were performed using a LC-ESI-QqQ system (HPLC: 1200 Infinity Quaternary LC System (Agilent); Column: Accucore™ Polar Premium (2.6 µm, 150 mm x 2.1 mm i.d., Thermo Fischer Scienctific Inc.); QqQ: API 4000 Q trap (ABSCIEX)). In addition, Cholesterol flux and gene expression studies, as well as fecal microbiome profiling were performed.

After gavage of radiolabeled cholesterol, we observed a decrease in plasma and an increase in liver and BAT cholesterol indicative of an accelerated cholesterol flux. Notably, we found a 6-fold increased hepatic expression of genes responsible for the alternative pathway of BA formation accompanied by a 50-fold elevation of BAs in the liver and the feces. Elevated fecal BA levels were dependent on functional hepatic lipoprotein clearance by the Ldlr, ApoE, and Lrp1, and prevented by the cholesterol uptake inhibitor ezetimibe. 16S rRNA sequencing of the fecal microbiome revealed a distinct clustering pattern dependent on host housing conditions and fecal BA availability.

Despite higher cholesterol uptake as a consequence of increased food intake, a systemic BAT-mediated program maintains cholesterol homeostasis by increased hepatic BA synthesis as well as fecal BA excretion. Hence, we assume a functional interplay between altered host BA levels, energy metabolism, and gut microbiota.

On mass ambiguities in shotgun lipidomics

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Mass spectrometry-based Lipidomics aims to identify as many lipid species as possible from complex biological samples. Due to the large combinatorial search-space unambiguous identification of lipid species is far from trivial. Mass ambiguities are common in direct-injection shotgun experiments, where an orthogonal separation (e.g. liquid chromatography) is missing.
Using the rich information of available lipid databases, we generated a comprehensive rule set describing mass ambiguities, while taking into consideration the resolving power (and its decay) of different mass analyzers. Importantly, common adduct species and isotopic peaks are accounted for and are shown to play a major role, both for perfect mass overlaps due to identical sum formulae as well as resolvable mass overlaps. We identified known and hitherto unknown mass ambiguities, while also ranking lipid classes by their propensity to cause ambiguities.
Based on this new set of ambiguity rules, guidelines and recommendations for experimentalists and software developers of what constitutes a solid lipid identification in both MS and MS/MS can be formulated.
For researchers new to the field, our results are a compact source of ambiguities, which should be accounted for. These new findings also have implications for the selection of internal standards, optimal choice of instrument resolution, and sample preparation for example in regard to adduct ion formation.

Micro-arrays of faux tissue to investigate ion suppression effects in MALDI mass spectrometry of lipids

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P 18 **PRICE**

MALDI mass spectrometry imaging (MSI) is used increasingly to visualize the molecular distribution of lipids and other biomolecules in tissue sections. More recently, various QMALDI techniques were developed for quantitative imaging, typically by using internal standards. The largest complication in MALDI-MSI of lipids results from ion suppression effects. To obtain a deeper insight into ion suppression phenomena under MSI conditions, fundamental studies are needed. Here, we developed artificial lipid-free "faux tissues" (FT) as substrates that enable a controlled adjustment of the lipid composition by spiking, while mimicking essential characteristics of soft tissue.

In order to measure FTs spiked with up to 24 different phospholipid concentrations in one imaging run, a micro-array mold with vials of 2 mm x 5 mm, filled with 20 µL of spiked FT material, was constructed. At -22°C cryo-sections (20 µm) of the micro-array block were prepared and subsequently desiccated at $5 \cdot 10^{-3}$ mbar for 8 min. For MALDI-MSI the sections were coated with 2,5-dihydroxybenzoic acid (DHB) matrix by sublimation/recrystallization. MSI data was recorded with a QStar (AB Sciex) and a Synapt G2-S HDMS (Waters) mass spectrometer.

A set of gel-like substances was tested as a carrier for FT. Important criteria for their suitability to mimic soft biological tissue were their water content, texture, stiffness, handling of the material, and its applicability for MALDI-MSI. Considering all factors, best results were obtained with carboxymethyl-cellulose. In following preparative experiments, the FT carriers were spiked with different amounts of a phospholipid-mix to generate tissues containing a lipid concentration comparable to brain tissue. After optimizing the matrix coating protocols for lipids, we then investigated the ion suppression effect of phosphatidylcholines (PC) on other phospholipid classes by varying the PC content of the FT and recording the ion signal intensities of all detected lipid signals in dependence of the spiked lipid concentration.

Lipid profiling of the obligate biotrophic fungus *Rhizophagus irregularis* during mycorrhization of *Lotus japonicus*

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Arbuscular mycorrhiza (AM) formation is an ancient and widespread symbiosis between plants and fungi of the *Glomeromycota*. AM formation is one of the most important symbioses for agricultural performance of crop plants. The fungi form a vast network of hyphae in the soil and supply their host plants with water and nutrients. Plants benefit from this interaction especially during growth under water- and phosphate-limiting conditions. In turn, the obligate biotrophic AM fungi are dependent on their host plants for carbon supply. AM fungi have previously been noted for their accumulation of large amounts of storage lipids. Palmitvaccenic acid (16:1) has been described as a marker fatty acid to determine the amount of AM fungi in the soil. However, a comprehensive lipid profiling with modern tools of lipidomics analysis was not available prior to this work.
Lipid profiling was performed mainly by direct infusion nanoflow liquid chromatography mass spectrometry on an Agilent 6530 Q-TOF MS with nanospray infusion ion source technology. Lipid extraction and purification protocols were adapted to analyse different lipid classes. The lipid profiling was aided by gas chromatography (GC) MS analysis and thin layer chromatography (TLC).
We studied the accumulation of triacylglycerol, diacylglycerol, phospholipids, galactolipids, sterols, and sphingolipids during the colonization of *Lotus japonicus* roots with *Rhizophagus irregularis* (syn. *Glomus intraradices*). We determined several molecular species of phospholipids and triacylglycerols, which can serve as markers for fungal membrane lipids or storage lipids. The composition of fungal phospholipids, but not that of triacylglycerol, changes during intraradical growth. This is likely a prerequisite for symbiosis, achieving functional compatibility between the fungal and the plant membranes. Data mining in genomic and transcript databases revealed the absence of a gene encoding multidomain fatty acid *de novo* synthase in the sequence of the fungus.

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Comparative lipidomics of human colorectal cancer

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Colorectal cancer (CRC) is one of the most common and deadly cancers in the world. It originates from the epithelial cells lining the colon or rectum when the balance between cell death and proliferation is perturbed. Colorectal tumors can grow into the intestinal wall invading nearby tissue, spread to lymph nodes, and metastasize to other organs, where the extent of which are used to describe the pathologic stage of CRC (i.e. TNM staging system). The progression of colorectal carcinogenesis has long been associated with the disturbed lipid metabolism, for instance the involvement of lipids in cell proliferation, apoptosis, and inflammation that highly increases the risk of carcinogenesis. Thus, characterizing the lipidome of colorectal tumors could benefit biological functional studies and may confer new insights into the mechanisms of CRC pathophysiology.
Here, we conducted a pilot study on colorectal tumors and peripheral healthy tissues from 40 CRC patients with matched age, gender, and body mass index. The pathologic stage of CRC was determined by the TNM system. A shotgun lipidomics analysis was performed to quantify approximate 400 lipid species from 13 subclasses for comparative studies. Levels of several lipid subclasses were changed significantly in colorectal tumors, including phosphatidylethanolamines (PE), phosphatidylinositols (PI), ether/ester-linked phosphatidylcholine (PC-O), lysophosphatidylcholines (LPC), lysophosphatidylinositols (LPI), ceramides (Cer), sphingomyelins (SM), and cholesteryl esters (CE). In addition, diacylglycerols (DG), phosphatidylcholines (PC), phosphatidic acids (PA), ether/ester-linked PE, and lysophosphatidylinositols (LPI) were associated closely with the TNM staging of CRC. Taken together, our results suggest that a broad range of lipids may contribute to the colorectal carcinogenesis.

Platelet Extracellular Vesicles are carriers of lipids and proteins involved in vascular- and neurodegenerative diseases

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Introduction: During activation and senescence, platelets release increased amounts of platelet extracellular vesicles (PL-EVs). We established a platelet storage lesion *in vitro* model for size, proteomic, lipidomic, and transcriptomic characterization of PL-EVs over 5 days in platelet concentrates.
Methods: After 5 days, PL-EVs were isolated by filtration and differential gradient ultracentrifugation into 5 PL-EV subfractions (PL-EV F1-F5) and exosomes (PL-EXs) and subjected to Nanoparticle Tracking Analysis, Flow Cytometry, proteomic/lipidomic mass spectrometry.
Results: F1-F2 (lowest density) show the highest content of free cholesterol (37% of platelet cholesterol) and express vesicular and endosomal sorting complex (ESCRT)-associated protein. Subsets F1-F2 are also enriched in CD62P, Annexin V, and alpha-synuclein. Subsets F2-F4 show the highest content of CD63 and LAMP-2. Amyloid-beta precursor protein (APP) and the apoptotic lipid 7-ketocholesterol peak in subsets F3-F4. Subsets F3-F5 are enriched in caveolin-1, apolipoproteins (apo) -I, -J, and –E. Subset F4 is enriched in peroxisomal enzymes and plasmalogens to enable prostanoid synthesis. Subset F5 (high density) contains mitochondrial proteins and a high content of lysophosphatidic acid (LPA), phosphatidylserine (PS), ceramide (Cer), phosphatidylglycerol, and cardiolipin (CL). PL-EXs are enriched in lipid-raft and adhesion markers. Addition of HDL3/apoA-I during storage significantly reduce PL-EVs by 52%, correlating with the concentration of added apoA-I.
Conclusions: Different lipid and protein compositions determine PL-EVs. Certain PL-EVs might represent autophagic vesicles and PL-EXs resemble lipid rafts. Segregation of alpha-synuclein and amyloid beta precursor protein, ApoE/J in less dense, and dense PL-EVs respectively, indicate a role in neurological disease. The high content of PS, Cer, LPA, CL, and mitochondrial proteins in F5 relates this EV-subset to mitochondria. HDL3/apoA-I reduce PL-EV release during platelet senescence, improving intracellular lipid processing/vesicle transport, and cholesterol efflux.

Assessing the limit of quantification in the analysis of lipid mediators by LC-MSMS

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Bioactive lipid mediators participate in the regulation of the inflammatory processes and there is considerable interest in the measurement of these lipids. It is best practice to validate analytical methods using an appropriate biological matrix, however for many lipid mediators, this is a challenge as they are present as endogenous species. In this study, we have compared different methods of calculating the Lowest Limit of Quantification (LLOQ) for a range of lipid mediators using LC-MS/MS. Both signal-to-noise and accuracy and precision were calculated with authentic standards together with standards spiked into human plasma. Our results show that there is a considerable variation in LLOQ values determined by different analytical approaches. Specifically, the method was less sensitive when LLOQ values were obtained using accuracy and precision in contrast to signal-to-noise measurements. By way of exemplar the LLOQ for 15-hydroxyeicosatetraenoic acid (15-HETE) was 2 pg and 4 pg on column for the signal-to-noise and accuracy and precision methods respectively. Further, the LLOQ was higher when lipid mediators were spiked into plasma as a result of high endogenous concentrations of these species in the matrix. We conclude that the LLOQ determined using authentic standards is a good indicator of instrument performance, whereas standards spiked into a matrix are more representative of the biological milieu.

Lipidomic methods to unravel the impact of the microbiome on host lipid metabolism

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The gut microbiota is a complex ecosystem, with its composition and diversity depends on various factors including diet, environment, health, and disease. Our preliminary data indicate strongly that gut microbiota influence host lipid metabolism.
Electrospray tandem mass spectrometry (ESI-MS/MS) offers an excellent platform to quantify lipid species with high sample throughput. Major glycerophospholipid and sphingolipid classes are accessible by direct flow injection of crude lipid extracts. Whereas low abundant or isobaric species require frequent liquid chromatographic separation coupled to tandem mass spectrometry (LC-MS/MS). Lipid species quantitation is applicable for biomarker search in large clinical studies as well as basic research in a variety of sample materials including plasma, lipoprotein fractions, cells, tissues, and faeces. Moreover, these methods provide insight into dynamics of the lipid species metabolism by administration of stable isotope labelled precursors or lipid species. For example major pathways of the glycerophospholipid metabolism may be profiled using D_9-choline, D_4-ethanolamine, and $^{13}C_3$-serine; labelled acetate and fatty acids may be applied to profile fatty acid synthesis, uptake, and metabolism.
Taken together, mass spectrometry offers a powerful tool box to study the influence of gut microbiota on host lipid metabolism including resorption of fatty acids, lipid synthesis, and storage. Moreover, lipidomic analyses of faecal samples provide insight into lipid profiles of the microbiome and its impact on intestinal lipid modification.

Development of a flow injection-high resolution MS method to analyze and quantify the lipid composition of human plasma and adipose tissue

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The lipid profile of the human plasma and adipose tissue is an emerging biomarker to compare the state of physiological and pathological conditions. Excess lipids or defects in lipid storage are associated with diseases such as metabolic syndrome, which results from complex interactions between genetic and environmental factors, including the gut microbiota.

Electrospray flow injection analysis high resolution mass spectrometry (ESI-FIA-HR-MS) offers an excellent platform to quantify lipid species with high mass resolution and high sample throughput. The composition of major neutral lipid, glycerophospholipid, and sphingolipid classes can be directly accessed from crude lipid extracts. In adipose tissue, the analysis of phospholipids is hampered by a vast amount of triglycerides. Therefore, we are developing a two-step lipid extraction method to improve the detection and quantification of polar lipid classes in fat. A first step with apolar organic solvent recovers neutral lipids. A second extraction allows to collect the remaining lipid classes and allows a more detailed analysis of polar lipids (e.g. glycerophospholipids) due to a decreased amount of triglycerides.

In summary, ESI-FIA-HR-MS offers a high-throughput method to analyze and quantify lipid species profiles of human plasma and adipose tissue. A two-step extraction of triglyceride-rich samples significantly improves the detection of polar lipids after removal of neutral lipids.

Inflammation in different adipose tissue depots during human cancer cachexia and its impact on fatty acid modifying enzymes

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Background: Systemic inflammation and loss of fat mass are hallmarks of cancer cachexia. Adipose tissue is capable of pronounced synthesis of inflammatory molecules.
Aim: To investigate inflammatory pathological changes and their impact on fatty acid modifying enzymes in different adipose tissue depots in cancer cachexia.
Methods: Volunteers were recruited after signing the informed consent form at the University Hospital. Patients subjected to incisional hernia surgery were included in the control group (Control), while gastrointestinal carcinoma patients were divided into a weight-stable cancer (WSC) group and a cachectic cancer group (CC). Cachexia was diagnosed as described in [1]. Inflammatory factors of different fat depots (subcutaneous adipose tissue [SAT] and visceral adipose tissue [VAT]) and plasma were measured with Luminex xMAP technology. In cell culture 3T3-L1, U937 and THP cells were challenged with cytokines, and the enzyme expression was measured using real-time PCR.
Results: In SAT several pro-inflammatory interleukins were diminished significantly in WSC compared to the control. In both, SAT and VAT, the CC group exhibited higher IP-10 concentrations compared to the controls. IL-8 was increased significantly in CC compared to controls in VAT ($p = 0.022$) as well as in plasma ($p \leq 0.0001$). Furthermore, IL-8 and IP-10 VAT contents correlated with plasma cytokine concentrations in CC. FADS2 expression in VAT of CC was increased compared to Control while ELOVL5 and ELOVL2 tended to be reduced during cancer cachexia. Elongase and desaturase expression was modulated in U937, THP, and 3T3-L1 cells by stimulation with inflammatory cytokines.
Conclusion: Changes of the inflammatory profile observed in plasma, SAT, and VAT could have a direct impact on enzymes involved in fatty acid desaturation and elongation in cancer cachexia. This could influence lipid mediator formation and explain changes in the fatty acid profile found in cachectic patients.

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LCPUFA supplementation as a means to reduce to retinopathy of prematurity in extremely preterm infants

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Around 1-2% of infants are born extremely preterm, i.e. born before 28 weeks of gestation. Extreme preterm birth does not only increase the risk of neonatal death, but is also connected to a number of long-term complications including both developmental and sensory impairments. One such complication is retinopathy of prematurity (ROP), a disease caused by poor neurovascular development of the retina that may result in reduced vision or in severe cases lead to blindness.

Due to infants born extremely preterm miss the third trimester of gestation *in-utero*, they also miss the supply of nutrients, hormones, and other factors provided normally at appropriate via the placenta. Instead, they rely on parenteral nutrition for the first weeks of life. Parenteral lipid solutions given to preterm infants often completely lack or have only low concentrations of the long chain polyunsaturated fatty acids (LC-PUFA) docosahexaenoic acid (DHA) and arachidonic acid (ARA). It has been shown in multiple studies that blood levels of DHA and ARA decline rapidly after birth in preterm infants.

We are investigating, if supplementation of LC-PUFA to extremely preterm infants starting from birth can reduce the frequency and severity of ROP and other morbidities. Preliminary results and future perspectives will be presented.

Development of a robust high-throughput high-resolution LC-MS/MS analysis method for faecal sterols and cholesterol metabolites in human stool

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Currently, there is a lack of understanding about the importance of the gut microbiome's role in health in general, and in detail of its modification effect on the stool lipidome. Emerging evidence suggests that the human gut microbiota plays diverse physiological roles that regulate energy balance, brain development, and function, thereby determining our risk of developing brain and diet-related disorders.
Cholesterol and its oxidation products reveal a major effect on the cardiovascular health and thereby play a key role in the stool lipidome. Plant sterols and stanols have significant impact on the intestinal cholesterol absorption and give, together with cholestanol, information about the intestinal sterol absorption. Bile acids are the final degradation products of the cholesterol metabolism. Accompanied with cholesterol, they form mixed micelles and facilitate sterol and lipid absorption in the intestine. Certain gut bacteria species possess capability to modify free bile acids and its conjugates, which effect the bile acid profile in the intestine. Until the present day, the impact of modified intestinal bile acid profile is in the focus of research.
Added complication in fecal sterol analyses arise from the stool sample material itself. Stool is a complex sample material with wideranging analyte concentrations, lipid modifying enzymes, and heterogeneous lipid distribution within the matrix. Therefore, a highly robust and representative analytical method is crucial for reproducible fecal sterol and cholesterol metabolite analysis. Here, a short, reliable, and specific liquid chromatography–high-resolution mass spectrometry method (LC-MS/HR-MS) will be developed for the quantification of ring and side-chain oxidized cholesterol, cholesterol precursors, plant sterols, and stanols and bacterial modified cholesterol.

Labeling and identification of lipidated proteins during adipogenesis

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Because of the increasing prevalence of obesity, adipogenesis has become an intensely studied developmental process, with attention to various aspects of fat cell biology. Lipid modifications have turned out to be important regulatory entities in diverse signalling events. To gain a better understanding of this during the development of fat cells (adipogenesis), we decided to take a look at the fate of fatty acids. To this end, we synthesized a modified palmitic acid containing a terminal alkyne. This modification does not interfere with the cellular metabolic pathways and it can be utilised to introduce a detection tag by bio-orthogonal chemistry.
In a first series of experiments, we have taken a look at the saturated fatty acid palmitate. In an adipogenesis model, where OP9 cells undergo differentiation into fat cells under the influence of rosiglitazone (1 µM), we observed first an increase and later a decrease in fatty acid-modified proteins with increasing incubation time of the rosiglitazone. Basic hydrolysis conditions only removed a small part of the fatty acid labelling, indicating that the majority is not only located on the side chains of cysteine residues.
We are currently undertaking an enrichment and identification protocol using chemically cleavable linkers in order to identify the targets of the palmitate PTM and their modification sites.

Studying cellular signaling with ultra-high temporal resolution using a µMixing µFluidic device

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Cellular signaling is highly complex and dynamic and involved biomolecules are often targets in the pharmaceutical industry. Although signaling occurs within seconds to milliseconds, conventional cell stimulation methods used in the laboratory involving tubes and manual pipetting are neither sufficiently precise nor reproducible to reliably studying such fast and also early signaling events.
Therefore, we propose the use of microfluidics to achieve reproducible cell stimulation experiments at high temporal resolution. A PDMS micromixer microfluidic device, monolithically integrating a first mixing segment connected to second via a stimulation zone, was developed to conduct all experimental steps required: (1^{st}) mixing of cells with stimulation solution, (2^{nd}) control the stimulation duration, and (3^{rd}) lyse cells and quench signaling. For the set of flow rates and micromixer geometry used, full mixing of solutions was achieved in 80 ms. Different stimulation time points were performed using different devices in order to study EGF-induced protein phosphorylation signaling in EGFR-overexpressing HEK293 cells. Cells and EGF solution were injected at the first mixing segment and lysis buffer was injected at the second mixing segment. The flow through containing the cell lysate was collected for mass spectrometry-based quantitative phosphoproteomics. Preliminary data revealed EGFR autophosphorylation already after 0.5 s, while most downstream signaling occurred after 1-5 s. Importantly, our device does not only allow studying temporal protein phosphorylation profiles, but will also pave the way for time-resolved lipid signaling studies.

Development of a Nano-LC-MS System for global lipidomics

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Lipids are key players of cellular systems that are particularly known for their roles in structural compartmentalization and energy storage. Furthermore, they are also able to fulfill signaling functions in biological systems at marginal concentrations. In order to identify low abundant lipids such as mediators, analytical methods need to be developed to allow an accurate characterization and quantification of these biomolecules with high sensitivity and specificity. In comparison to conventional LC-MS analyses, nanoLC-MS systems promise significant higher measurement sensitivity, reduced sample consumption, and decreased solvent usage. For this reason, we developed a nanoLC-ESI-MS method for lipid analyses. Different column materials, flow rates temperatures and sample loading conditions were compared to investigate their impact on nano-scale reverse phase chromatography. The reproducibility and robustness of the method was shown through repetitive separations of a yeast extract on the developed nanoLC-MS system. Furthermore, the lipid detection sensitivity of the nano-LC system was benchmarked to a corresponding narrow-bore LC system.

Identification of phospholipid species implicated in dementia by untargeted high resolution LC-MS and data dependent MS/MS

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Alzheimer's Disease (AD) is the leading cause of dementia in the elderly. Recently, Mapstone *et al.* reported a panel of plasma phospholipids that predicted cognitively normal adults who later progressed to either mild cognitive impairment or dementia due to AD (Mapstone 2014). This study used a targeted metabolomics p180 kit (Biocrates, Life Science AG, Austria) that measures phospholipids by infusion and selected reaction monitoring (SRM) with a triple quadrupole mass spectrometer. Because phospholipids have many isomers and isobars comprised of different combinations of fatty acids and alkyl/alkenyl ethers, it is not possible to assign unequivocally the phospholipid species using this low resolution approach. Identification of the fatty acid molecular identity of the phospholipids implicated in AD is critical for two reasons: (a) one needs to determine the functions of these phospholipids and their contributions to pathophysiology of the disease; and (b) one needs to determine the molecular identity before one can develop quantitative assays to measure theses phospholipids in human plasma.
The objective of this study is to determine fatty acid constituents of seven phosphatidylcholine (PC) lipids: PC 36:6, PC 38:0, PC 38:6, PC 40:1, PC 40:2, PC 40:6, and PC 40:6e (ether) in plasma by using liquid chromatography and high-resolution accurate mass and tandem mass spectrometry (LC/MS/MS).

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Quality control for lipid mediator quantification

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Lipid mediators are bioactive derivatives of ω-3 and ω-6 polyunsaturated fatty acids. They play a crucial role in the signaling network during microbial infection and mark the inflammation status of the host. The identification and quantification of PUFAs and their derivatives by liquid chromatography - tandem mass spectrometry (LC-MS2) enables a comprehensive insight into the regulation of immune cell activities. The golden standard for such analyses are triple quadrupole mass spectrometers utilizing multiple reaction monitoring with only unit resolution.
In this study, we applied parallel reaction monitoring (PRM) using Q-TOF and QExactive Plus mass spectrometers. We analyzed 36 reference compounds on both instrument platforms to determine how spectral comparisons should be computed to implement a quality control. We can show in many cases that chosen quantifier ions are not specific for one mediator because of the high number of isomeric structures. We developed a spectral comparison score that takes into account matches for m/z values as well as the general intensity profile. Because of the advantage of the high resolution mass spectrometry, we incorporated a filter that ensures that only fragments with the compositional constraint of CnH3/2n-yOz ($n = 4, 5, 6 \ldots 22$; y: $0, 1, 2$; $z = 0, 1, 2 \ldots 5$) are considered. Intensities of matched signal were compared with Spearman's rank correlation, to incorporate the spectral profile as quality criteria.
The developed algorithm was capable to score structural related molecules with high values but would still be sufficient to distinguish it from the identical LM. False discovery rates were suitable to underscoring the advantage of high resolution mass spectrometry. Finally, we developed a software that allows to 1) process raw peak lists, 2) generate customized spectral libraries, 3) choose specific quantifier ions and 4) perform spectral comparisons. The software is platform independent and can help to improve the quality control for lipid mediator quantification.

Attachment and modification of phosphorylcholine residues in teichoic acids of *Streptococcus pneumoniae*

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Streptococcus pneumoniae has a unique nutritional requirement for exogenous choline and attaches phosphorylcholine (P-Cho) residues to the N-acetylgalactosamine (GalNAc) moieties of its teichoic acids (TAs). Two different enzymes, LicD1 and LicD2, mediate the attachment of P-Cho at the O-6 positions of the two GalNAc residues, which are present in each pneumococcal TA repeating unit. LicD1 is essential and assumed to incorporate the P-Cho residues at the α-D-GalNAc. LicD2 is a non-essential enzyme likely transferring another P-Cho residue to the β-D-GalNAc. The P-Cho residues are important for the anchoring of surface-localized choline-binding proteins (CBPs) at the pneumococcal cell wall. Until now, no structural proof for the specific attachment of P-Cho to β-D-GalNAc by LicD2 has been furnished. In this study, we assessed this by isolating LTA from the pneumococcal mutant strains TIGR4ΔcpsΔlicD2 and TIGR4ΔcpsΔpceΔlicD2. The detailed structural analysis by ^{31}P NMR and mass spectrometry proved the specific attachment of P-Cho residues to β-D-GalNAc by LicD2.
Furthermore, the role of the pneumococcal phosphorylcholine esterase (Pce) in the modification of pneumococcal TAs has been elucidated. It is known, that *in vitro* Pce hydrolyzes about 15-30% of the total P-Cho residues attached to pneumococcal TAs. In order to clarify the specificity of the Pce-mediated P-Cho hydrolysis, different concentrations and pH conditions for the treatment of isolated LTA with purified Pce have been explored. For these investigations, fully P-Cho decorated LTA isolated from *Streptococcus pneumoniae* TIGR4ΔcpsΔpce was treated with different concentrations of heterologously produced Pce. The changes in the P-Cho substitution pattern depending on the different Pce concentrations were investigated by mass spectrometry and 31P NMR. We could clearly reveal that *in vitro* Pce hydrolyses the P-Cho residues at β-D-GalNAc residues as well as at the terminal α-D-GalNAc. By contrast, *in vivo* studies reveal that only the two P-Cho residues of the terminal repeating unit are affected.

Comprehensive lipidomics approach for platelet analysis

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Platelets are the central building block of coagulation and hemostasis and linked directly to or affected by several metabolic diseases. In platelets, a ceramide/acid sphingomyelinase (ASM) system has been found to play a crucial role in cell signalling and membrane structure rearrangement [1-2]. It is known that ASM catalyzes the sphingomyelin hydrolysis to generate ceramides in response to cellular stress. The deficiency of ASM causes Niemann-Pick disease type A and B. Thereby, lipids could be key elements in these processes but so far, a comprehensive lipid profile of the ASM knockout in mice platelet is still missing. To obtain a deep view into the ASM platelet lipidome, we developed multiple lipidomics approaches to detect and quantify lipids with high sensitivity and accuracy.
Platelet pellets were collected from ASM knockout and control animals and the lipid extracted using a methyl-tert-butyl ether (MTBE) extraction procedure. To obtain a comprehensive understanding of how lipids are regulated after platelet activation, we developed a reliable identification and quantification approach. Therefore, we initially optimized different lipid extraction strategies, established a selected reaction monitoring (SRM) based LC-MS/MS workflow, and tailored a shotgun lipidomics approach for the lipid analysis in platelets. The obtained data were evaluated with adapted Skyline [3] or with LipidXplorer [4].
In total, more than 28 lipid classes including around 200 lipid species were identified tentatively and quantified in mice platelets. Among these lipid classes, cholesterol, phosphatidylethanolamines, phosphatidylserine, and sphingomyelin were found as the most abundant lipid classes while lactosylceramide were found at very low amounts. By applying the targeted LC-MS/MS methods with fast polarity switching, we were able to identify and quantify 59 sphingolipids in mice platelets. Our preliminary results indicate the potential benefits of a deep lipidome profiling to understand molecular mechanisms in disease relevant model systems such as the ASM knockout mouse.

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A new highway between targeted and non-targeted lipidomics

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Skyline, a powerful software primarily designed for proteomics applications, was extended successfully to targeted LC-MS/MS lipidomics [1]. This proof-of-principle study provided a systematic workflow for the straightforward method design and analysis of selected reaction monitoring (SRM) data in lipidomics based on lipid building blocks. To strengthen the targeted lipidomics workflow by providing a user-friendly interface for lipid researchers, we developed a tool named "LipidCreator", which has several features including i) using lipid building blocks, ii) latest lipid nomenclature, iii) lipid fragmentation, iv) transition mass-producer, and v) the direct communication with Skyline. Because Skyline is a vendor independent platform, it supports SRM data from triple quadrupole mass spectrometry and parallel reaction monitoring (PRM) data from Orbitrap high resolution mass spectrometry for interpretation and visualization. PRM data evaluation is different from SRM because PRM generates MS2 spectra, which require a library for comparison. Therefore, we established an *in silico* spectral library for lipid species by computing fragments from known lipid fragmentation patterns. This *in silico* spectral library is not only used for PRM data inspection, but will also be the foundation for further data independent acquisition (DIA) analysis.

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Systems biology of the unfolded protein response in glioma

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Diffuse gliomas are one of the most common types of human brain tumor and among the most deadly of all human cancers. Depending on the tumor stage, they can be extremely invasive and resistant to different kinds of cancer therapies. Recent studies reveal that the unfolded protein response (UPR) network might be responsible for the tumor proliferation and therapeutic resistance. Due to this reason, the UPR network has become a potential target for the cancer treatment aiming to induce cancer cell death by activating the UPR apoptosis site. Moreover, it has already been suggested in various studies that on the one hand, the alteration in membrane lipid composition can induce UPR and on the other hand, UPR can affect the lipid metabolism of the cell. Our goal is a systematic characterization of the UPR network to identify new therapeutic targets for gliomas. For this, we use different human glioma cell lines as well as neural progenitor cell model systems from genetically modified mice carrying mutations relevant to glioma pathogenesis, such as loss of Pten or mutation of Idh1. We combine different systems biology approaches to analyze these model systems on the transcriptional, translational, proteomics, and lipidomics level. From the functional studies performed on the ER stress induced human and mouse model systems (HEK293 and NIH-3T3 cell lines), we observed an enhancement at the protein and mRNA level of several UPR proteins, which indicated the activation of the UPR pathway in response to the treatment with Tunicamycin to induce ER stress. Followed by the proteomics study, we quantified relatively 2000-3000 proteins and observed the activation of the UPR pathway through the activation of its downstream factors. The network analysis of the proteomics data from human and mouse model systems present the pathways, which are enriched or depleted significantly. Quantitative lipidomics analysis will be carried out to investigate the effect of membrane lipid composition on UPR and how UPR can alter the cellular lipid metabolism. The potential marker proteins we identify in these experiments will then be analyzed and validated in human glioma specimens after computational modeling and integration with the results that obtained in the earlier modeling approaches in regard to the ER stress - and unfolded protein response.

Fragmentation behavior of neuronal lipids by HCD-MS/MS

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Biological membranes provide hydrophobic barriers to separate the interior of cells and cellular compartments from their outer environment. This separation, however, requires the transport of material and information through the membranes, a process often carried out by the proteins and protein complexes residing in the membranes. Transfer of information can also be accomplished by membrane fusion, e.g. in neuronal synapses during neurotransmitter release into the synaptic cleft. Membrane fusion is usually triggered by the proteins residing in the membranes and their interplay with specific lipids. However, the role of the lipids in these processes is only poorly understood and a complete description of the membranes is therefore of fundamental importance to gain insights into these processes.
Mass spectrometry evolved as a valuable tool for comprehensive lipid identification in complex samples mostly by their specific fragmentation patterns. We studied the fragmentation behavior of various lipid classes found in neurons, including phosphatidylethanolamines, Phosphatidylcholines, Phosphatidylserines, Phosphatidylinositols, and Sphingolipids. Mass spectra were acquired at high resolution using high energy collisional dissociation (HCD) on a Q-exactive plus Orbitrap mass spectrometer. Lipid structures were characterized by employing different energies and polarities producing diagnostic fragmentations ions. Collecting mass spectra of standard lipids delivers information on the characteristic fragmentation behavior of specific lipid classes allowing the global analysis of natural membranes in neurons.

Lipid profile as diagnostic marker for antimycobacterial treatment monitoring

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Detection of active tuberculosis (TB) remains a challenge in pre-clinical and clinical trials of TB. The low sensitivity of sputum smear test and weeks needed for results from the more sensitive method of culture methodology test makes it an urgent need for a fast reliable diagnostics of TB.
Therefore, using lipidomics approach, we search for lipid molecules that can be used as diagnostic markers during TB therapy. Additionally, there are currently nomarkers reflectingantibiotic efficacy and completion of the therapy by clearance of MTB at site of infection.
Therefore, differences in lipid profiles between MTB genotypes Haarlem, Beijing, Uganda, EAI, WA2 and Canetti were initially studied by shotgun lipidomics and high level of Phosphatidylinositol (PI) was observed in all analyzed lineages. PI (35:0) was the most abundant lipid specie in all genotypes and showed the expected fragment ions in negative ion mode for fatty acidswith FA (16:0) with m/z of 255.2, FA (19:0) with m/z of 297.3 referred as tuberculostearic acid (TSA) and thePI head group with m/z of 241.0.We screened several biomaterials for this molecule like plasma, plasmaperipheral blood mononuclear cell (PBMC), cell cultures from both mouse and human origin and detected only negligible amounts.PI (35:0) was therefore targetedas anovel marker molecule to count MTB in cell culture system avoiding the time consuming CFU determination. We can show that already 10,000 MTB are detectable with this approach. The currently applied MS method is sensitive enough to detect PI and TSA in presence of $3 \cdot 10^5$ macrophages infected with 10^5 bacteria with no false positive results from 36 cultures.
Taken together, the analysis of PI (35:0) by ESI/MS is a promising approach to empower TB research in cell and animal models. We will further investigate how antibiotic quantitation and quantification of PI (35:0) can be integrated into one workflow.

Monitoring induced changes in the sphingolipid pathway upon inducing insulin sensitivity and resistance in different tissues by mass spectrometry

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Adipocytes are well known for their essential role as energy storage depots for triglycerides. However, data emerging over the past several years have established an additional role for the adipocyte, a secretory cell. Adipocytes express and secrete numerous peptide hormones and cytokines, including TNF-α which has been proposed as a link between obesity and insulin resistance because such circulating factors selectively induce enzymes that promote sphingolipid synthesis [1]. Studies in cultured cells and isolated tissues implicate sphingolipids in cellular events associated with diseases such as insulin resistance, diabetes and cardiovascular disease. This realization raises many possibilities for additional links between adipose function and insulin resistance, independent of the adipocyte's roles in energy storage and release.
Ceramides are membrane lipids which are involved in obesity. There has been work showing that inhibiting the ceramide synthesis can improve insulin sensitivity and reduce the effect of insulin resistance in rodents [2]. For this reason we wanted to investigate the sphingolipid synthesis/signaling pathway which is responsible for ceramide synthesis in the context of protein regulation upon inducing insulin resistance and insulin sensitivity in different cell culture models of different tissues. To achieve this, we took hepatocytes, myocytes and neurons into culture and treated them with rosiglitazon (insulin sensitivity) and TNF-α (insulin resistance) and a combination of both, to investigate effect on sphingolipid synthesis and signaling.
To screen and identify the regulation of the sphingolipid pathway, we first set up a label free quantitative global proteomics approach by using a high resolution mass spectrometry system (QExactive Plus). We used STRING 10 and Cytoscape 3.3.0 to visualize the regulated proteins, pathway and network dynamics.
In total we could identify 44 proteins in myocytes, 36 proteins in neuron cells and 33 proteins in hepatocytes which are part of the sphingolipid metabolism (108 proteins in total). Our preliminary results reveal a different regulation pattern of proteins involved the sphingolipid synthesis, dependent on the tissue. While the treatment with TNF-α shows results to be expected for myocytes (25 proteins out of 44 get upregulated) it showed the complete opposite for hepatocytes (7 proteins out of 36 get upregulated) in the context of sphingolipid metabolism. For those cells, an upregulation of proteins involved in the sphingolipid pathway was only seen with a combination of a treatment with rosiglitazon and TNF-α (26 proteins get upregulated).

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Identifying modulations in cancer lipid metabolism using a lipidomics approach

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Lipid metabolism is aberrantly regulated in more than half of human cancers, and these changes are considered to support the malignant behavior. However, detailed knowledge on the modulations occurring in cancer lipid metabolism is still limited. By generating an overview of the metabolic modulations in cancer, this project aims to shine light on novel therapeutic targets.
Lipid metabolism takes place at all cellular membranes and involves >1 000 lipid species. The numerous enzymes that constitute lipid metabolic pathways are compartmentalized differentially within the cell, forming an uneven distribution of lipids and a unique "lipid fingerprint" of each membrane. As lipids are essential components of cell structure and signaling, lipid metabolism partakes in determining membrane physiological function and enables spatiotemporal control of cellular processes.
Lipidomics emerges as a powerful tool for quantitative global lipid profiling. Our hypothesis is that lipidomics needs to be performed at the subcellular levels to efficiently illuminate changes in lipid metabolism due to the complex spatial organization of metabolic pathways. This project will systematically chart cancer-induced metabolic modulations by developing a method that couples affinity purification of individual membranes to mass spectrometry-based lipidomics. The method will be used to perform comparative analyses of the subcellular lipidomes of oncogene-transformed vs. non-transformed cell lines. Consequently, the generated lipidomics datasets will be used to build models on the modulations in lipid metabolism occurring in cancer cells. Ultimately, this can be used to identify novel targets of cancer therapy.

SIMPLEX reveals myoglobin as a key player in cardiac lipid metabolism

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Cardiovascular diseases are the number one cause of death worldwide. In many forms of heart disease, including diabetic cardiomyopathies, heart failure and ischaemic heart disease, alterations in cardiac mitochondrial energy metabolism contribute to a decrease in cardiac efficiency and to contractile dysfunction. Moreover, elevated deposition of fatty acids into intramyocardial lipids is associated with distinct pathological processes that impair cardiac function. Even though these processes are still elusive, more light sheds on with the recent knowledge on myoglobin's (Mb) function. Previous studies support Mb as a protector of cardiac function in hypoxia and myocardial ischemia/reperfusion-injury [1].
We applied the previously developed extraction protocol SIMPLEX (**SI**multaneous **M**etabolite, **P**rotein, **L**ipid **EX**traction procedure) [2] on the cardiac muscles from an Mb-/- mouse model as well as the wild-type littermates. The mass spectrometry based lipidome analysis reveals a significant increase in triglycerides with a preferential incorporation of palmitic and oleic acids. The 3-fold higher deposition of lipids in Mb-/- hearts was associated with depressed cardiac function compared to wild-type mouse hearts as assessed by echocardiography. Glycerophospholipid levels as well as the metabolome and proteome related to FA metabolism tend to be unaffected by myoglobin ablation. Our results have important implications to pathophysiological conditions where lipotoxicity is evident.

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