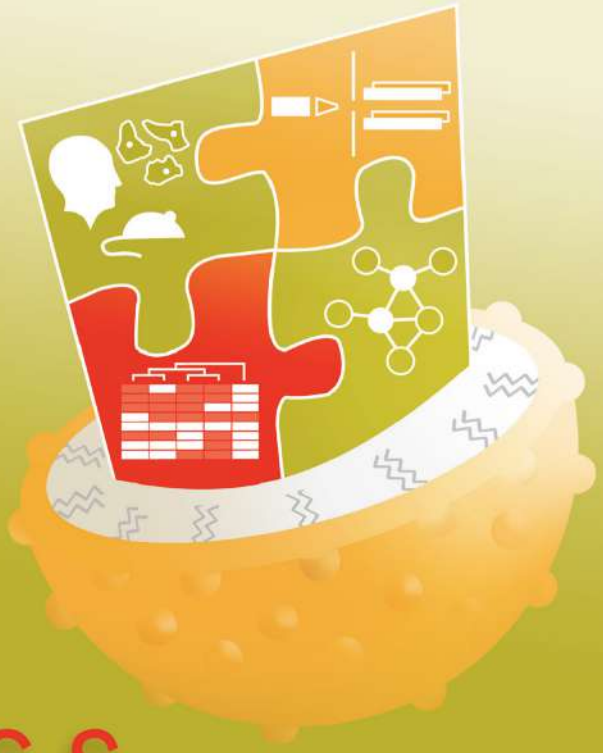

LIPIDOMICS FORUM

11. - 13. November 2018

VENUE

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

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Robert Ahrends	ISAS
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Nicolas Gisch	RCB
Dominik Schwudke	RCB
Dominik Kopczynski	ISAS

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Sunday | **November 11**

10:00 - 17:00 **WORKSHOPS**

16:00 **REGISTRATION OPENING**

18:00 **OPENING SESSION** | Chair: Albert Sickmann

Ole G. Mouritsen

T 01 **Gastrophysics for lipid physicists**

Department of Food Science, University of Copenhagen, Copenhagen, Denmark 10

19:30 **DINNER**

20:30 **WINE TASTING**

Dr. Kopczynski's travel through German wine regions

Monday | **November 12**

8:45 - 9:30 **TUTORIAL** | Chair: Philipp Westhoff

Gerhard Liebisch

T 02 **Guidelines for Lipidomics Analysis and Reporting – the Lipidomics Standards Initiative (LSI)**

Institute of Clinical Chemistry and Laboratory Medicine, University of Regensburg, Germany . . . 11

9:30 - 10:15 **KEYNOTE** | Chair: Philipp Westhoff

Michal Holčapek

T 03 **Supercritical fluid chromatography – mass spectrometry: novel approach for high-throughput lipidomics**

University of Pardubice, Czech Republic 12

10:15 – 10:30 **COFFEE BREAK**

10:30 – 12:10 **SESSION 1** | Chair: Cristina Coman

10:30 Josef Ecker

T 04 **Gut microbiota-derived short chain fatty acids are precursors for hepatic lipid synthesis**

Lehrstuhl für Ernährungsphysiologie, Technische Universität München (TUM), Freising, Germany 13

10:50 Madhumita Chatterjee

T 05 **Platelet Lipidome Influences Thrombotic Disposition: Implications for Coronary Artery Disease and Regulation by CXCL12-CXCR4-CXCR7 Axis**

Department of Cardiology and Cardiovascular Disease, University Clinic Tübingen 14

11:10 Denise Wolrab

T 06 **Ultrahigh-Performance Supercritical Fluid Chromatography Hyphenated with Mass Spectrometry: A powerful tool for lipidomic quantitation in clinical samples**

University of Pardubice, Department of Analytical Chemistry, Pardubice, Czech Republic. 15

11:30 PRIZE Marcus Höring

T 07 **Application of stable isotope labeling high resolution mass spectrometry to study the intestinal lipid metabolism**

Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Germany 16

11:50 Maria Fedorova

T 08 **Adipose tissue lipidomics – from analytics to metabolic networks**

Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Leipzig, Germany 17

12:10 – 13:00 **LUNCH**

13:00 – 13:45 **KEYNOTE** | Chair: Philipp Westhoff

Ivan Tancevski

T 09 **Mediators: From bench to bedside**

Innsbruck Medical University, Innsbruck, Austria 18

13:45 – 14:30 **KEYNOTE** | Chair: Philipp Westhoff

Valerie O'Donnell

T 10 **Regulation of abdominal aortic aneurysm by enzymatically-oxidized phospholipids critically depends on tissue localisation**

Division of Infection and Immunity, Cardiff University, Cardiff, Wales 19

14:30– 14:45 **COFFEE BREAK**

14:45 – 15:30 **KEYNOTE** | Chair: Philipp Westhoff

Thorsten Hornemann

T 11 **1-Deoxy-Sphingolipids - When Sphingolipids Become Headless**

UniSpital Zurich, University Zurich, Switzerland 20

15:30 – 15:45 **CONFERENCE PHOTO**

Lecture Hall

15:45 – 18:30 **POSTER SESSION**

Santosh Kapil Kumar Gorti

P 01 **HILIC LC-MSMS assay for high-throughput targeted lipdomics analysis**

SCIEX, Framingham, MA, USA 27

Dietrich Merkel

P 02 **Differential Mobility Separation DMS-based separation of bile acid isomers**

AB Sciex Deutschland GmbH 28

J.-W. Lackmann

P 03 **Forehead Lipid Profiling Reveals Oxidizing Capacity of kINPen Treatment**

ZIK plasmatis at Leibniz Institute for Plasma Science and Technology 29

PRIZE Cristina Coman

P 31 **SIMPLEX: from extraction to underlying molecular mechanisms in cardiomyopathy**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Otto-Hahn-Str. 6b, 44227 Dortmund, Germany 57

Hans-Frieder Schött

P 32 **A multi-omics approach to screen for modified hippocampal lipid signaling pathways triggered by lifestyle conditions**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Otto-Hahn-Str. 6b, 44227 Dortmund, Germany 58

Maceler Aldrovandi

P 33 **The temporal generation of oxidized phospholipids and fatty acids during physiological blood clotting reveals complex interconnected networks, controlled by cells and enzymes**

Systems Immunity Research Institute, School of Medicine, Cardiff University, UK 59

PRIZE Niklas Danne-Rasche

P 34 **Nano-LC/NSI MS refines lipidomics by enhancing lipid coverage, measurement sensitivity, and linear dynamic range**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Otto-Hahn-Str. 6b, 44227 Dortmund, Germany 60

PRIZE Patricia Rodrigues

P 35 **Regulation of lipid metabolism by GATA6: an integrated 'omics approach**

Systems Immunity Research Institute and Division of Infection and Immunity, School of Medicine, Cardiff University, Cardiff, CF14 4XN, UK 61

PRIZE Lisa Hahnefeld

P 36 **Biomarker research and short time chromatography: a utopia**

Institute of Clinical Pharmacology, pharmazentrum frankfurt/ZAFES, Goethe University, Frankfurt am Main 62

Nils Hoffmann

P 37 **Lipid Compass**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Otto-Hahn-Straße 6b, 44227 Dortmund, Germany 63

PRIZE Bing Peng

P 38 **Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome**

Leibniz-Institut für Analytische Wissenschaften-ISAS-e.V., Dortmund, Germany 64

18:30 - 18:40 **BUS TRANSFER TO DINNER**

Meeting point in front of ISAS main entrance

19:00 – open end

CONFERENCE DINNER

At Lokalmanufaktur, Friedensplatz 1, 44135 Dortmund

Tuesday | November 13

8:45 – 9:30

TUTORIAL | Chair: Bing Peng

Rainer Lehmann

T 12 **QC in clinical sample preparation - Anything to consider before starting high resolution lipidomics investigations**

Clinical Chemistry Central Laboratory, Department of Diagnostic Laboratory Medicine, University Hospital Tuebingen, Germany 21

9:30 – 10:30

Open discussion - Challenges in clinical lipidomics

Olga Vvedenskaya, Robert Ahrends, Gerhard Liebisch

10:30 – 10:45

COFFEE BREAK

10:45 – 12:05

SESSION 2 | Chair: Bing Peng

10:45 Schweiger-Hufnagel, Ulrike

T 13 **CCSPredict: Using a machine learning approach for higher confidence in Lipid identification**

Bruker Daltonik GmbH 22

11:05 Carsten Jaeger

T 14 **A comprehensive data-independent acquisition strategy for UPLC-QqTOF-MS lipidomics combining SWATH and MS^E**

Charité - Universitätsmedizin Berlin 23

11:25 Zhixu Ni

T 15 **Integration of high-throughput lipidomics data into genome-scale metabolic model of adipose tissue.**

Institute of Bioanalytical Chemistry, Faculty of Chemistry and Mineralogy, Universität Leipzig, Leipzig, Germany 24

11:45 Denes Turei

T 16 **lipyd: A Python module for lipidomics LC tandem MS data analysis**

Joint Research Center for Computational Biomedicine, University Hospital RWTH Aachen 25

12:05 – 12:50

KEYNOTE | Chair: Bing Peng

Martin Giera

T 17 **Dehydrocholesterol reductase 24, a key enzyme for the integration of cholesterol and lipid mediator biosynthesis**

Leiden University Medical Center, Leiden, Netherlands 26

12:50 – 13:10

CLOSING SESSION

Awards for best posters and talks

13:10

FAREWELL LUNCH PACKS

Gastrophysics for lipid physicists

Ole G. Mouritsen[1]

[1]Department of Food Science, University of Copenhagen, Copenhagen, Denmark

T01

The history of physics demonstrates repeated moves into other areas turning them into physics. This has happened with, e.g., astronomy, geology, chemistry, as well as biology and is possibly about to happen with gastronomy, turning it into gastrophysics. The subject matters of gastrophysics are food, preparation techniques, and flavor; and the methods of study include the arsenal of modern techniques and concepts from the physical sciences. Our interaction with the world, including food we eat, proceed via the five senses, one of which is taste proper. However, our general perception and appreciation of 'taste arise as the brains multimodal integration of all sensory inputs folded with memories, expectations, emotions, etc. In my talk I will give an overview of the physiology, neurogastronomy, and gastrophysics of taste and exemplify the presentation with the science behind a specific basic taste, umami, and the tactile component of taste, mouthfeel. On the way we will see how biophysics and interface science come in all the way from the membrane receptor level to the texture of food.

[1] O. G. Mouritsen and K. Styrbæk. Umami: Unlocking the Secrets of the Fifth Taste. *Columbia University Press, New York*, 2014.

[2] O. G. Mouritsen and K. Styrbæk. Mouthfeel: How Texture Makes Taste. *Columbia University Press, New York*, 2017.

Guidelines for Lipidomics Analysis and Reporting – the Lipidomics Standards Initiative (LSI)

Gerhard Liebisch[1]
John A. Bowden[2], William J. Griffiths[3], Robert Ahrends[4], Todd W. Mitchell[5], Makoto Arita[6], Masanori Arita[7], Christer S. Ejsing[8], Michal Holčapek[9], Markus Wenk[10], Kim Ekroos[11]

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[2]Chemical Sciences Division, Hollings Marine Laboratory, National Institute of Standards and Technology, USA
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[5]School of Medicine, Illawarra Health and Medical Research Institute, University of Wollongong, Wollongong, NSW 2522, Australia
[6]Laboratory for Metabolomics, RIKEN Center for Integrative Medical Sciences (IMS), Tsurumi, Yokohama, Kanagawa 230-0045, Japan
[7]RIKEN Plant Science Center, Yokohama, Japan, Department of Biophysics and Biochemistry, The University of Tokyo, Tokyo, Japan
[8]Department of Biochemistry and Molecular Biology, VILLUM Center for Bioanalytical Sciences, University of Southern Denmark, DK-5230 Odense, Denmark
[9]Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic
[10]Singapore Lipidomics Incubator (SLING), Department of Biochemistry, YLL School of Medicine, National University of Singapore, Singapore, Singapore
[11]Lipidomics Consulting Ltd., FI-02230 Esbo, Finland

T02

Mass spectrometry (MS)-based lipidomics has revolutionized lipid research. The ability to identify and quantify numerous lipid species in cells, tissues and biofluids is currently drawing major attention to this technology. However, an increasing number of studies are reporting poor quality lipidomics data with misidentification and inaccurate/inappropriate quantification of lipid molecules.

Therefore, we have launched the Lipidomics Standards Initiative (LSI; https://lipidomics-standards-initiative.org), an open-access web-resource to both describe and promote guidelines for minimal acceptable lipidomics data quality. LSI covers the main analytical lipidomics approaches; shotgun, LC-MS and imaging. It aims to present guidelines for the analytical steps of traditional lipidomics workflows including how to 1) collect and store samples, 2) extract lipids, 3) perform mass spectrometric analysis, 4) perform data processing, and 5) data reporting. This further covers method validation, including the critical steps for reliable quantification.

Currently, the LSI web-resource is established with a first set of guidelines for the most applied lipidomics workflows i.e. application of low resolution MS/MS and high resolution MS in shotgun, RPLC, NPLC/HILIC and imaging. It contains a collection of isomeric and isobaric overlap as well as in-source fragmentations of lipid species which may lead to potential misidentifications. To stimulate consistent data reporting, it summarizes and exemplifies the rules for annotation of lipid species. Additionally, LSI aims to provide links to reference materials and to provide channels for discussion and further development of these standards.

LSI introduces urgently needed guidelines for lipidomics, leading to improved data quality and common language for data comparison and exchange.

Supercritical fluid chromatography – mass spectrometry: novel approach for high-throughput lipidomics

Michal Holčapek[1]
Denise Wolrab[1], Ondřej Peterka[1], Robert Jirásko[1]

[1]University of Pardubice, Czech Republic

T03

Numerous lipid molecules and present in eukaryotic cells, and they fulfill various physiological functions. The dysregulation of lipidomic pathways is often related to serious human diseases, such as cancer, cardiovascular diseases, diabetes, *etc*. The complexity of lipids results in the fact that multiple analytical methods are needed to cover a broader range of lipidome. Accurate, comprehensive and high-throughput methods are required for the analysis of large sample sets in the clinical research, which is challenging for analytical techniques due to the enormous complexity of lipid structures. Two well-established approaches are commonly used in lipidomics, such as shotgun and LC/MS, both having some advantages and limitations. Now we have developed a new high-throughput and comprehensive approach for analysis of lipids in biological samples using ultrahigh-performance supercritical fluid chromatography (UHPSFC) followed by ESI-MS identification and quantitation.

Total lipid extracts from selected human tissues and body fluids (plasma and urine) are prepared by chloroform – methanol – water extraction. UHPSFC experiments were performed on UPC^2 instrument (Waters) using 1.7 μm particle bridged ethylene hybrid silica column, separation temperature 60°C and gradient of methanol – water – ammonium acetate mixture as a modifier coupled with Synapt HDMS G2Si instrument (Waters) using electrospray ionization. Individual parameters of UHPSFC analysis are carefully optimized to achieve a maximum number of separated lipid classes. Final UHPSFC method enables a fast separation up to 28 nonpolar and polar lipid classes within 6 min analysis including the partial separation of species inside individual classes. Mass spectra with high mass accuracy and high resolving power are acquired using ESI in both positive- and negative-ion modes for the identification of individual species. The quantitative analysis is performed using internal standards for each lipid class added during the extraction step. The comparison with established shotgun MS approach and MALDI-MS will be shown as well. All methods are fully validated in line with FDA and EMA recommendations for high-throughput clinical quantitation, such as tumor and surrounding normal tissues, body fluids from cancer patients and healthy volunteers. Our software LipidQuant is used for the semi-automated data processing, and then concentrations of lipids are statistically evaluated using multivariate data analysis (MDA) methods. This methodology has been applied to cancer research and the differentiation of healthy and cancer groups based on the lipidomic analysis of over thousand people.

This work was supported by project No. 18-12204S sponsored by the Czech Science Foundation.

Gut microbiota-derived short chain fatty acids are precursors for hepatic lipid synthesis

Josef Ecker[1]
Gerhard Liebisch[2], Alida Kindt[3], Thomas Clavel[4], Dirk Haller[5], Claudine Seeliger[1], Jan Krumsiek[3]

[1]Lehrstuhl für Ernährungsphysiologie, Technische Universität München (TUM), Freising, Germany
[2]Institute of Clinical Chemistry, Universitätsklinikum Regensburg, Regensburg, Germany
[3]Institute of Computational Biology, Helmholtz Zentrum München, Neuherberg, Germany
[4]Institute of Medical Microbiology, Universitätsklinikum Aachen, Aachen, Germany
[5]Lehrstuhl für Ernährung und Immunologie, Technische Universität München (TUM), Freising, Germany

T04

Short-chain fatty acids (SCFA) are generated by fermentation of complex carbohydrates by the gut microbiota before they reach the circulation via the portal vein. Although it is known that SCFA are potent signaling molecules, their role for host *de novo* lipid synthesis is unclear.

A comprehensive multi-omics view of the impact of intestinal microbial colonization on hepatic lipid metabolic processes in mice identified that the gut microbiota induce generation of saturated and mono-unsaturated fatty acids and phospholipids in the liver. By supplying mice with stable isotope labelled precursor SCFA, i.e. ^{13}C-acetate, via oral gavage, we show that SCFA generated in the gut lumen are precursors for host lipid synthesis. ^{13}C-enrichment in hepatic C16 and C18 fatty acids was calculated using mass isotopomer distribution analysis (MIDA) after mass spectrometric analysis. Dietary intervention experiments under conventional and gnotobiotic housing conditions demonstrated that levels of *de novo* synthesized fatty acids depend on SCFA concentrations in portal blood, dietary fiber content and gut microbiota composition.

Together, these findings demonstrate that the presence of gut microbiota promotes hepatic lipid synthesis and metabolism by providing a significant amount of SCFA as precursors for generation of C16 and C18 fatty acid containing phospholipids. These interactions between the gut microbial ecosystem and host lipid homeostasis are essential for general physiology, disturbances might be associated with pathophysiological conditions including metabolic diseases.

[1] Kindt A, Liebisch G, Clavel T, Haller D, Hörmannsperger G, Yoon H, Kolmeder D, Sigruener A, Krautbauer S, Seeliger C, Ganzha A, Schweizer S, Morisset R, Strowig T, Daniel H, Helm D, Küster B, Krumsiek J, and Ecker J. The gut microbiota promotes hepatic fatty acid desaturation and elongation in mice. *Nature Communications*, Accepted Article, 2018.

Platelet Lipidome Influences Thrombotic Disposition: Implications for Coronary Artery Disease and Regulation by CXCL12-CXCR4-CXCR7 Axis

Madhumita Chatterjee[1]
Jörg Schlotterbeck[2], Johannes Rheinlaender[3], Malgorzata Cebo[2], Dominik Rath[1], Meinrad Gawaz[1], Tilman E. Schäffer[3], Michael Lämmerhofer[2]

[1]Department of Cardiology and Cardiovascular Disease, University Clinic Tübingen
[2]Institute of Pharmaceutical Sciences,Universität Tübingen
[3]Institute of Applied Physics, Universität Tübingen

T05

Hyperlipidaemia propagates thrombosis, while activated platelets in acute coronary syndrome (ACS) patients show increased platelet oxLDL binding. Platelet surface expression of the chemokine CXCL12 and its cognate receptors CXCR4-CXCR7 are elevated in ACS, having significant prognostic impact. Our current study explores the platelet lipidome in coronary artery disease (CAD) patients, the atherothrombotic consequences of platelet lipid metabolism and the influence of CXCL12-CXCR4-CXCR7 axis on platelet-lipid association.

Platelet-oxLDL is enhanced in CAD patients, particularly so in ACS patients with angiographic evidence of intracoronary thrombi. *Ex vivo* analysis of intracoronary thrombi suggest platelet as a significant source of lipid deposition in intracoronary thrombi. Comprehensive untargeted lipidomic analysis (UHPLC-ESI-QTOF-MS/MS analysis of the lipid extract was carried out on an Agilent 1290 UHPLC instrument hyphenated to a Sciex TripleTOF 5600+ hybrid mass spectrometer) of platelets from CAD patients reveal enhanced intraplatelet oxidized phospholipids, cholesteryl-esters, elevated sphingomyelin-ceramides, acylcarnitine, PLA2(lysoPC), PLC(diacyglycerol) metabolites in ACS(STEMI) patients. Intra-platelet lipidome is greatly influenced by platelet activation status and significantly regulated by anti-platelet medications like aspirin. Previously pharmacological CXCR7 agonist was shown to lower plasma cholesterol in hyperlipidaemic *Apoe-/-* mice to ameliorate atherosclerosis. Currently we have observed that platelet oxLDL levels in CAD patients correlates positively with CXCR7 surface expression, a receptor which also mediates platelet lipid uptake in presence of CXCL12. However, the pharmacological agonist acting through CXCR7 can counteracts LDL/oxLDL induced reactive oxygen species (ROS), mitochondrial superoxide generation, (per)oxidative lipid modifications. CXCR7 agonist also counteracts activation induced release of pro-inflammatory and pro-thrombotic lipid mediators (thromboxane, arachidonic acid, 12-HETE, ceramides). LDL/oxLDL induces α-granule degranulation, $\alpha_{IIb}\beta_3$-integrin activation, apoptosis (thrombogenic phosphatidylserine exposure, mitochondrial membrane depolarization), triggers dynamic shape change, aggregation, thrombin generation, thrombus formation *ex vivo* and following administration in mice. Pharmacological CXCR7 agonist acting as an anti-platelet mediator can counteract LDL/oxLDL induced thrombotic response. These evidences suggest the potential of therapeutic strategies directed through CXCR7 might influence intraplatelet lipid metabolism and its thromboinflammatory consequences.

Ultrahigh-Performance Supercritical Fluid Chromatography Hyphenated with Mass Spectrometry: A powerful tool for lipidomic quantitation in clinical samples

Denise Wolrab[1]
Ondřej Peterka[1], Robert Jirásko[1], Roman Hrstka[2], Michal Holčapek[1]

[1]University of Pardubice, Department of Analytical Chemistry, Pardubice, Czech Republic.
[2]Masaryk Memorial Cancer Institute, Regional Centre for Applied Molecular Oncology, Brno, Czech Republic.

T06

Lipids are biologically active compounds with several biological functions. They are involved in energy storage, function as signaling molecules and present constituents of cell membranes. A dysregulation is often related to serious diseases, e.g., various types of cancer. The analysis and quantification of lipids in biological samples, like human plasma or serum, may allow the differentiation of healthy and diseased donors. However, the chromatographic analysis is challenging due to the structural and chemical diversity as well as concentration differences of lipids in biological samples. Ultrahigh-performance supercritical fluid chromatography (UHPSFC) allows a fast separation of nonpolar and polar lipid classes and consequently also the quantitation by employing an internal standard for each lipid class and mass spectrometry (MS) detection. Liquid-liquid extraction was employed for the extraction of lipids from human serum. UHPSFC/MS measurements were carried out on an Acquity Ultra Performance Convergence Chromatography (UPC^2) system coupled to a hybrid quadrupole traveling wave ion mobility time of flight mass spectrometer Synapt G2 Si from Waters (Milford, USA). Lipid classes were separated according to their polar head groups employing an Acquity BEH UPC^2 column in gradient mode. Positive ion ESI mode in the resolution mode was employed for analysis of lipid species belonging to glycerophospholipids, sphingolipids, and glycerolipids. For validation purpose, we determined LOD, LOQ, matrix effect, recovery, repeatability as well as reproducibility for 8 lipid subclasses using appropriate exogenous internal standards for each lipid subclass. Results show slight differences in the method performance parameters depending on the nature of the head group of the lipid subclass. Furthermore, we developed a workflow for monitoring, method performance and sample preparation quality among the study in order to ensure reliable data quality. Therefore, a QC sample, which is a representative mixture of serum or plasma samples, spiked with the internal standard were regularly measured, and the online measurement control was performed by extracting the peak areas of internal standards for each sample. The data processing for identification and quantification was performed by using a homemade software tool based on Microsoft Excel called LipidQuant. An embedded database allows the identification by comparing the measured m/z with the accurate m/z values. The quantification of the lipid species is achieved via the corresponding internal standard. The method was applied for the lipid analysis of human serum and plasma samples of cancer patients and healthy volunteers. Multivariate data analysis (MDA), such as nonsupervised principal component analysis (PCA), supervised orthogonal partial least square discriminant analysis (OPLS-DA), S-plots, and box plots, allowed the visualization and identification of lipid profile differences between healthy and diseased donors. This methodology is the subject-matter of a pending patent application. This work was supported by GAR 18-12204S (Czech Republic).

Application of stable isotope labeling high resolution mass spectrometry to study the intestinal lipid metabolism

Marcus Höring[1]
Josef Ecker[2], Emma Slack[3], Christian Wolfrum[4], Gerhard Liebisch[1]

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[2]Institute of Nutritional Science, Technical University of Munich, Germany
[3]Institute of Microbiology, Swiss Federal Institute of Technology Zurich, Switzerland
[4]Institute of Food Nutrition and Health, Swiss Federal Institute of Technology Zurich, Switzerland

T07 **PRIZE**

Fatty acids (FA) are key molecules for the assembling of various lipid classes. The required FAs can be obtained through cellular de novo synthesis or by resorption of dietary lipids. Therefore the dietary lipids are emulsified by bile acids (BA) and hydrolyzed by lipases in the lumen. After FA uptake into the enterocytes, they are reassembled mainly into glycero(phospho)lipids and released into the circulation. Although it is known that gut microbiota influences the BA profile, it is still unknown whether gut microbiota also affects FA resorption in mammalians.

Aim of this work is to apply a flow injection high resolution mass spectrometric (FIA-HR-MS) method to study the intestinal lipid metabolism and the influence of gut microbiota on these processes with stable isotope labeled FAs. The deuterated FAs were applied to mice by oral gavage of D_5-FA 16:0 (palmitic acid) and D_{93}-TG 48:0 (tripalmitin). At various time points mice were sacrificed and different sections of the intestine, liver and blood samples collected. Lipids were extracted in the presence of non-endogenous internal standards and subjected to quantitative FIA-HR-MS.

In first experiments, the incorporation of perdeuterated palmitic acid could be detected in various TG (triglyceride) and PC (phosphatidylcholine) species. The most abundant species found in the small intestine are D_{31}-TG 52:3, D_{31}-TG 52:2, D_{31}-PC 34:2 and D_{31}-PC 34:1. In plasma we could additionally detect the signals of D_{31}-TG 50:1 and D_{31}-PC 36:4. Labeled species showed time-dependent profiles reflecting the lipid flux from gut to circulation followed by hepatic uptake and release. These data also relate to labeled FA profiles determined by GC-MS. In addition, the investigation of germ-free (GF) and specific pathogen-free (SPF) mice revealed different amounts of deuterated FAs detected downwards the intestine assuming an increased FA resorption in GF mice.

In summary, feeding of stable isotope labeled FAs followed by quantitative FIA-HR-MS and GC-MS offers a valuable tool to trace intestinal FA uptake and metabolism. Ongoing experiments in GF mice will provide further understanding how gut microbiota influences these processes.

Adipose tissue lipidomics – from analytics to metabolic networks

Maria Fedorova[1,2]
Zhixu Ni[1,2], Georgia Angelidou[1,2], Mike Lange[1,2]

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

T08

Obesity has reached epidemic proportions in modern societies with a prevalence of more than 20% of the population and has been recognized as a risk factor for numerous metabolic disorders including type 2 diabetes (T2D), cardiometabolic, liver and renal diseases. Onset and disease progression are closely associated with metabolic reconfiguration of white adipose tissue (WAT) showing the important role of lipids and lipid related metabolites in WAT expansion and development of the disease. Thus, a systems wide study on lipid metabolic and regulatory functions in insulin sensitive and resistant obese patients can significantly contribute to the understanding of disease mechanisms.

Using a combination of analytical, bioinformatics and modeling techniques, we are aiming to provide systems medicine view on the development of obesity associated insulin resistance. Using combination of differential extraction, chromatography separation and high-resolution mass spectrometry, adipose tissue lipidomics atlas is being reconstructed to provide the scaffold for integration of WAT lipids in genome scale metabolic models (GEM). Currently over 1000 molecular lipid species have been identified and relatively quantified in subcutaneous and visceral WAT of insulin sensitive and resistant obese patients. Those data are used for the enrichment of exciting GEMs in lipid-centric metabolic pathways, and in combination with publicly available transcriptomics data as well as proteomic results obtained in our laboratory will allow identification of metabolic alterations associated with diseases onset and development.

Mediators: From bench to bedside

Ivan Tancevski[1]

[1]Innsbruck Medical University, Innsbruck, Austria

T09

Atherosclerosis is still the leading cause of death in industrialized countries, and novel therapies to modulate cholesterol metabolism are urgently needed. Additionally, any approach promoting resolution of inflammation within the arterial wall would be expected to protect from atherosclerosis and its clinical sequelae such as myocardial infarction and stroke. We have recently reported that arachidonic acid (AA)-derived bioactive lipid mediators including lipoxins (LXs) may be employed to beneficially influence whole body cholesterol homeostasis. Moreover, scientific evidence suggests LXs to promote plaque stability, both by counteracting leukotriene-mediated recruitment of monocytes and by affecting the phenotype of atherosclerotic plaque macrophages. In search of novel treatment options to treat and prevent atherosclersosis, we are currently working on LX-based approaches to beneficially affect both plasma cholesterol levels as well as monocyte biology. These therapeutic approaches include promotion of LX synthesis in the circulation by introducing lipoxygenase-expressing probiotics into the gut microbiome, and to study different Frp2/3 agonists in mouse models of atherosclerosis. We have recently unveiled that the AA metabolome plays a physiological role in whole-body cholesterol homeostasis in mammals, and hope that our on-going efforts will pave the way for the development of novel drugs based on the structure of AA metabolites, offering a novel therapeutic strategy to counteract cardiovascular disease in humans.

Regulation of abdominal aortic aneurysm by enzymatically-oxidized phospholipids critically depends on tissue localisation

Valerie O'Donnell[1]

[1]Division of Infection and Immunity, Cardiff University, Cardiff, Wales

T10

During the last 10 yrs we found that circulating blood cells and platelets generate hundreds of oxidized phospholipids, which we term eoxPL, several of which are required for blood clotting and are elevated in thrombotic disease. Here, we demonstrate a central role eoxPL in regulating AAA. Specifically, through activating coagulation, eoxPL either promoted or inhibited AAA depending on tissue localization. Ang II administration to *ApoE*$^{-/-}$ mice increased intravascular coagulation and promoted lesional eoxPL formation. Deletion of eoxPL-generating enzymes (*Alox12* or *Alox15*) significantly reduced AAA on a background of significantly dysregulated hemostasis. Intravenously-administered pro-coagulant PL caused clotting factor activation and depletion, induced a bleeding defect, and significantly reduced AAA development. This suggests that *Alox* deletion reduces AAA through diverting coagulation away from the vessel wall due to eoxPL deficiency, instead activating clotting factor consumption and depletion in the circulation. In mouse whole blood, ~44 eoxPL molecular species formed within minutes of clot initiation. These were significantly elevated with *ApoE*$^{-/-}$, and many were missing in *Alox*$^{-/-}$ mice, identifying the eoxPL that modulate AAA. Correlation network analysis defined sub-families related through oxylipin composition. Thus, eoxPL regulate AAA through complex interactions with coagulation. Thus, regulating the delicate balance between bleeding and thrombosis either within the vessel wall or circulation can either drive or prevent disease development.

1-Deoxy-Sphingolipids - When Sphingolipids Become Headless

Thorsten Hornemann[1]

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T11

1-DeoxySphingolipids (1-deoxySL) are atypical sphingolipids, which are formed due to a shift in the substrate preference of the serine palmitoyltransferase (SPT). 1-DeoxySL lack the C1 hydroxyl group of normal sphingolipids, which precludes their conversion to complexsphingolipids but also their further downstream metabolism.

The rare axonal neuropathy HSAN1 is caused by pathologically increased 1-deoxySL levels due to mutations in SPT. 1-DeoxySLs are toxic to neurons in culture, induce neurite retraction and disrupt neuronal structures, likely by interfering with regulatory components of the cytoskeleton. At increased L-serine concentrations, 1-deoxySL formation is significantly suppressed. HSAN1 mice, which received an L-serine enriched diet, showed normal 1-deoxySL levels and did not develop neuropathic symptoms.

1-DeoxySL are also elevated in type 2 diabetes and associated with beta cell dysfunction, wound healing defects and the diabetic sensory neuropathy (DSN). L-serine supplementation significantly improved neuropathy in diabetic rats.

Although 1-deoxySL cannot be degraded by the canonical sphingolipid catabolism, they appear to be metabolized by a set of CYP4F enzymes, which likely reflects a physiological detoxification mechanism. CYP4F expression is reduced in obesity whereas PPARα antagonists, such as fibrates, induce the expression of CYP4F enzymes and lower 1-deoxySL levels. CYP4F enzymes could therefore be a novel therapeutic target for DSN.

QC in clinical sample preparation - Anything to consider before starting high resolution lipidomics investigations

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T12

Lipidomics is more and more in use as a valuable tool for the investigation of blood and other body fluids in biomedical studies. Key for the success of those projects is the powerful combination of reproducible lipid extraction with comprehensive, high resolution mass spectrometry, and appropriate bioinformatics tools for data evaluation and visualization. However, even highly sophisticated lipidomics strategies will fail if pre-analytical steps of body fluid collection and/or handling are insufficient. In general the process of body fluid collection and handling is strictly controlled by detailed protocols or standard operating procedures (SOP), but pre-analytical errors, either systematic or accidental, cannot absolutely be prevented. Additionally, the choice of sample material (e.g. serum or plasma; first or second morning urine) may affect the outcome of lipidomics projects.

In this tutorial potential pre-analytical pitfalls of biomedical lipidomics projects will be illustrated. Recent results of our research investigating pre-analytical aspects of blood handling and processing are demonstrated. Suggestions for the design or modification of study protocols and SOPs are given. The following questions will be discussed: 1) Which are the most critical pre-analytical steps in biomedical studies?; 2) Is it possible to check the quality of clinical or biobank samples without having knowledge of the preceding processing, e.g. of blood handling and transportation?; 3) Which sample material is more suitable for high resolution analysis, serum or plasma?; 4) Are repetitive freeze and thaw cycles critical?; 5) Spot urine: what is the difference between first or second morning urine? Finally recommendations are presented for a line of action to plan and perform biomedical lipidomics projects aiming to analyze body fluids.

CCSPredict: Using a machine learning approach for higher confidence in Lipid identification

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T13

The use of ion mobility-featured mass spectrometers offers new options for higher confidence in annotations of target molecules. First, with the additional separation dimension compounds co-eluting from LC columns can be separated. The benefit is that a subsequent fragmentation will result in cleaner MS/MS spectra – crucial for any ID in lipidomics or other small molecule workflows. Moreover, ion mobility enables the determination of the collisional cross sections of ions. These values are specific properties for any ion species under given conditions (type of gas, pressure, temperature). Therefore, acquired values can be used for identification if they are compared to *in-silico* generated data or used in a library-based approach.

We present a new tool for the prediction of lipid CCS values. It is fully integrated in MetaboScape 4.0 and is based on a machine learning approach that was extended significantly to cover a wider range of lipid structures. Predicted values can be compared with the ones measured on a Bruker timsTOF Pro instrument. The Trapped Ion Mobility Spectrometry technology enables the exact measurement of CCS values at a very high reproducibility and TIMS resolving power. Both are critical pre-requisites to make full use of the increased confidence by CCSPredict. This workflow helps to assign the structural classes of lipids.

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A comprehensive data-independent acquisition strategy for UPLC-QqTOF-MS lipidomics combining SWATH and MS^E

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T14

Most lipidomic studies demand accurate lipid identification as well as precise quantitative information. Recent quadrupole-time of flight (QqTOF) mass spectrometers offer fast concurrent acquisition of MS^1 and MS^2 spectra, supporting robust compound annotation and precise peak ratio estimation (semi-quantification) in single runs. Sequential windowed acquisition of all theoretical fragment ion mass spectra (SWATH) and MS^E are two popular data-independent acquisition (DIA) schemes, differing, however, conceptually and in terms of achievable duty cycle, sensitivity and selectivity. Here, I will discuss advantages and disadvantages of the two schemes for applied lipidomics. Investigation of complex lipid samples (cell culture, blood) showed that SWATH yielded cleaner MS^2 spectra than MS^E and still sufficient MS^1 data points for precise quantification, but only when restricted to a narrow precursor mass range, e.g. m/z 600-1000 for common phospholipids. At a wider mass range (m/z 300-1200), associated longer cycle times (1 s) in SWATH diminished peak quality to a level affecting quantitative reproducibility. An analytical strategy was therefore developed combining the advantages of both DIA schemes. First, one or more SWATH methods are used to obtain a comprehensive set of high-quality MS^1/MS^2 spectral pairs. In a second step, these are translated to specific precursor-product ion pairs, which are quantified in a pseudotargeted fashion from a subsequent MS^E analysis. Benefits of this procedure against each of the individual methods alone and practical aspects (sample requirements, acquisition times) will be discussed and demonstrated for a large lipidomic study of mammalian cell lines.

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Integration of high-throughput lipidomics data into genome-scale metabolic model of adipose tissue.

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T15

Obesity and associated predisposition to type 2 diabetes have been actively studied over past few decades and onset of insulin resistance was closely correlated with a low-level chronic inflammation in expending adipose tissue (AT). As the major cell components of AT and one of the most insulin-responsive cell types, adipocytes are intensively studied by different approaches. The lipid metabolic pathways in adipocyte functional activities are important, since the dysregulation of adipocyte lipid metabolism and signal transduction was associated with proinflammatory tissue phenotype, AT disfunction and development of insulin resistance. Thus, a systems wide study of lipid metabolic and regulation functions in insulin sensitive and insulin resistant AT of obese patients can significantly contribute to the understanding of disease mechanisms. Previously published genome-scale metabolic models (GEMs) for adipocyte iAdipocytes1809 [1] provided a scaffold for further big data integration from different omics techniques. Here we used iAdipocytes1809 GEM as framework to integrate data from high-throughput lipidomics and proteomics analysis of insulin sensitive and resistance AT of obese patients. We extended and reconstructed the model into a lipidomics oriented network to analyze the metabolic and signaling function of different lipids and associated proteins. Enrichment and further GEM reconstruction has the potential to assist the early diagnostics of type 2 diabetes in obese patients using systems medicine approach.

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lipyd: A Python module for lipidomics LC tandem MS data analysis

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T16

The combinatorial and hierarchic diversity of lipid classes makes lipidomics data processing challenging. Software tools have been developed recently to address this challenge. However a library which could serve as a basis for development of data processing and analysis workflows is still missing.

Here we present lipyd, a Python module for lipidomics data analysis. lipyd consists of a number of submodules for various tasks. It comes with ~170 lipid varieties predefined, and users can add more, in order to build custom mass databases, in addition it incorporates SwissLipids and LipidMaps data. lipyd is able to search various adduct ion masses against all these data sets. The MS^2 module comes with ~90 types of aliphatic chain derived and ~150 headgroup derived fragments predefined. lipyd annotates MS^2 scans and applies rule based methods to identify lipids. The MS^2 spectrum identification is based on our own standards and literature. It covers more than 150 lipid varieties, most of them both in positive and negative ion modes. Beside the LC MS/MS analysis lipyd offers higher level methods to analyse features across multiple samples.

Python today is one of the most popular programming languages in bioinformatics and this ensures colleagues will find convenient to use lipyd and integrate it in their workflows. lipyd is a free, open source software available at github.com/saezlab/lipyd.

Dehydrocholesterol reductase 24, a key enzyme for the integration of cholesterol and lipid mediator biosynthesis

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T17

For many years, distal cholesterol biosynthesis, starting with the triterpene squalene has be seen as mere cholesterol production pathway. However, recent progress has assigned significant biological activities particularly to precursors found in distal cholesterol biosynthesis. For example has desmosterol, the ultimate Δ^{24}-unsaturated cholesterol precursor, been shown to selectively interact with and modify the responses of macrophages, $\Delta^{8(9)}$-unsaturated sterols have just recently been described for their crucial role in oligodendrocyte formation and remyelination and FF-MAS and T-MAS are important lipids during meiosis.

In this lecture we will focus on the role and possible drugability of the membrane bound enzyme dehydrocholesterol reductase 24 (DHCR24). DHCR24 has been proposed as possible drug target in diseases, ranging from hepatitis C virus infections to arteriosclerosis. We will discuss the development of effective and selective inhibitors of DHCR24 using a gas chromatography mass spectrometry based whole cell screening assay for target identification and selectivity assessment. We will subsequently discuss lipidomics based strategies for evaluating drug induced changes and actions as exemplified for one of our chemical probes. Starting from *in vitro* experiments followed by an *in vivo* assessment of the drug actions, this lecture will sketch a comprehensive picture how multiple chemical and biological methods can be used in combination, for deciphering the actions of a drug candidate. We will discuss phenotypic observations obtained with our optimized chemical probe, showing promising results in a murine zymosan A induced peritonitis model. We will discuss the observed drug effects and show how advanced lipidomics and metabolomics techniques were used in order to decipher the mechanism of action of the chemical probe under investigation, showing a possible relation between inhibiting distal cholesterol biosynthesis, the production of lipid mediators and related anti-inflammatory effects. Ultimately a comprehensive molecular picture of the obtained phenotypic findings based on the combined use of lipidomics, genomics, protein array analysis and a radioactively labeled probe mediated by an inhibition of DHCR24 will be presented.

HILIC LC-MSMS assay for high-throughput targeted lipdomics analysis

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P01

Introduction

There are about 150,000 different lipid molecular species present across the biological spectrum. 'Shotgun lipidomics is an established non-targeted approach for broad-based lipidomic analysis but it can suffer from inherent ion suppression and ambiguous identification. Herein we introduce a targeted lipidomics workflow with the combination of HILIC LC separation and multiple reaction monitoring (MRM) mass acquisition. HILIC LC chemistry provides reproducible isomer separation based on lipid classes. The Scheduled MRM^{TM} type data acquisition provides high sensitivity and wide coverage (over 1200 lipid species) for lipids screening and relative quantitation with 24 min run time. The method was extensively verified and is a robust solution for a targeted lipidomics workflow.

Methods

Standards and samples: Lipids standards (SPLASH heavy isotopic labeled standards, 17:1 standards and regular light standards) and bovine heart extract were purchased from Avanti and SCIEX. They were diluted with 50%:50%/MeOH: dichloromethane containing 10mM ammonium acetate before injection. **LC-MS**: Bovine heart extract samples were subjected to LC-MS/MS analysis with a SCIEX QTRAP® 6500+ mass spectrometer coupled with Exion HLPC system. A Waters XBridge Amide 3.5 µm, 4,6 x 150 mm column was used for HILIC based LC separation. A Scheduled MRM ^{TM} method was implemented to provide screening and relative quantification of over 1200 lipids with 24 min run time.

Preliminary Data

Minimization isomer interference among different lipid classes is known as one of major challenges for LC-MS/MS method development for lipidomics analysis. Very commonly, one lipid molecule can have multiple isomers in other lipid classes sharing the identical precursor and product masses. This cross-class crosstalk could be troublesome because mass spectrometers cannot differentiate these isomers based on MRM transitions. To overcome this issue, a HILIC chromatographic method is implemented to provide good LC separation based on lipid classes. In order to confirm separation efficiency, lipids standards as natural lipids extracts distinguished by lipid class (one class per standard) were injected individually to confirm that there was no isomer crosstalk among different lipid classes. Retention time reproducibility on a HILIC chromatography is very sensitive to changes of LC condition. A minor pH difference or organic composition change in the mobile phase might induce retention time shifts of analytes. To minimize retention time drift, extensive evaluation on mobile phase preparation was performed. A standard LC preparation protocol was developed to achieve optimal retention time reproducibility, which is especially critical for the Scheduled MRM ^{TM} method. Internal Standard from 19 different classes, which are either heavy isotopic labeled lipids or odd chain lipids, served as internal standard. This method provided extensive lipid class coverage including, CE, CER, DCER, HCER, LCER, TAG, DAG, MAG, LPC, PC, LPE, PE, LPG, PG, LPI, PI, LPS and PS.

Novel Aspect

HILIC-MS/MS by QTRAP® system provides a reliable solution for targeted lipidomics workflow with maximum sensitivity, wide coverage and high throughput.

Differential Mobility Separation DMS-based separation of bile acid isomers

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P02

Introduction
Bile acids are involved in a wide range of biological functions including lipid resorption, immunological functions and metabolic regulation. Through metabolic transformations, isomeric and isobaric variants are generated, which makes the unequivocal identification and quantification of individual chemical species difficult.

Here we introduce Differential Mobility Separation (DMS) as a methodology for the separation of bile-acid isomers. DMS is an ion mobility technology which separates molecules based on their dipolar moment. We show DMS separation in conjunction with chromatographic separation (LC-DMS-MS) as well as with direct infusion (DMS-MS). While the combination with chromatographic separation may improve selectivity, the separation power of SelexIon is sufficient for a clear separation of isomers, allowing for infusion-based fast quantification without the need for LC development.

Methods

Bile acid standards were prepared in 10% DMSO at a stock solution of 1 mM. Two groups were used: taurodeoxycholic acid (TDCA), taurochenodeoxycholic acid (TCDCA), tauroursodeoxycholic acid (TUDCA) with monoisotopic mass of (499,2967) and formula (C26H45NO6S) and glycodeoxycholic acid (GDCA), glycochenodeoxycholic acid (GCDCA) and glycoursodeoxycholic acid (GUDCA) with monoisotopic mass of (449,3141) and formula (C26H43NO5). For direct infusion, compounds were prepared in water:methanol 50:50, 0.1% FA at a concentration of 1 µM. For LC-separation preparation was done in the same solvent at a concentration of 0,1 nmol/ml with 1 µl injection volume. The samples were measured on a QTRAP® 6500 LC-MSMS system equipped with a SelexION® (DMS) device and coupled to an ExionLC system for the LC experiments.

Preliminary results

Using the DMS technology, we directly infused the bile acid standards individually and as mixtures to determine the CoV values of the different isomers. To this end, the compensation voltage was ramped over a range between -30V to 0V. The results showed almost baseline separation of the different isomers.

The next aim was to combine DMS with chromatographic separation. The combination of DMS with chromatography has several advantages: Firstly, DMS showed a marked reduction of chemical background for better quantification, resulting in improvement of the signal to noise ratio. A further advantage is less requirement for development of chromatographic separation, which was shown by the elimination of isomers from extracted ion chromatograms. This also eliminated the requirement for retention time assignment and less reliance on compound specific fragments which may be low abundant. For example, DMS allows to use a highly abundant fragment without the need for compound specificity.

Novel aspects

Differential mobility separation, increased selectivity of bile acid separation by LC-DMS-MS

Forehead Lipid Profiling Reveals Oxidizing Capacity of kINPen Treatment

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P03

Cold atmospheric plasmas (CAPs) have recently transitioned from laboratories into clinics. They offer various applications for treatments of skin diseases, such as chronic wounds [1]. However, basic research is lagging behind application, as many molecular interactions between plasma-generated reactive oxygen and nitrogen species (RONS) and their biological targets are not well understood. Due to the clinical applications, one major target are lipid membranes of eukaryotic cells, which form the first barrier of plasma-derived RONS. Due to the known critical interactions between ROS and lipids typically leading to lipid peroxidation, an in-depth understanding of these processes is required to assess the risks of CAP treatment and adjust current treatment regimes.

For this study, lipids were collected from healthy volunteers from pre-cleaned fore heads using glass slides. Samples were treated directly on the glass with the kINPen 09 using pure argon feed gas [2] for 45 s. After normalizing on the weight of collected samples, lipids were extracted with MTBE and lipid distribution measured for each volunteer using a high-resolution mass spectrometry. Nanospray infusion was realized by a Triversa-QExactive Plus setup in MS-DIA positive and negative mode. Acquired spectral data was analysed using LipidXplorer [3] with adapted mfql files.

Various lipid classes could be identified and similar lipid profiles could be obtained for most volunteers regardless of age or sex. After CAP treatment, increased amounts of oxidized lipid side chains [4] were observed for several lipid classes. Further investigation regarding the impact of plasma treatment conditions will allow adjusting current treatment regimens to either reduce involuntary damage to by-standing cells or increase production of relevant signalling molecules, such as prostaglandin.

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LC-MS^n semi-targeted method for comprehensive analysis of redox-lipidomes

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P04

Lipids are involved in different biological functions as building blocks of biological membranes, energy storage and cell signaling. Dynamic alterations of the lipidome, including lipid peroxidation, is a known hallmark of numerous human disorders associated with acute and chronic inflammation.

Lipid peroxidation is an enzymatic or non-enzymatic transformation of polyunsaturated fatty acids (PUFAs) leading to the formation of various lipid peroxidation products (LPPs) including low molecular weight aldehydes (i.e. 4-hydroxy-trans-2- nonenal, acrolein), truncated lipids (i.e. alkanals, alkenals), and hydroxy-alka(e)nals), isoprostanes, hydroperoxy-, hydroxy-, keto- and epoxy-derivatives. Numerous studies had shown the involvement of lipid peroxidation in the onset and progression of inflammatory based diseases such as diabetes, Alzheimer, Parkinson disease and cardiovascular diseases[1]. LPPs are known to modulate different cellular signaling pathways by inducing changes in the biological membranes[2], protein lipoxidation, and interaction with cell surface (e.g. scavenger receptors and TLRs)[3] and intracellular (e.g. PPARs) receptors[4].

Various biological activities of LPPs are mainly determined by their chemical diversity. In order to understand LPPs structure-functional relationships, specific and sensitive analytical tools allowing separation and identification of structural isomers are required. Liquid chromatography coupled on-line to mass spectrometry (LC-MS) allows high-throughput characterization of LPPs in biological samples. However, majority of the methods are not capable to distinguish LPP structural isomers.

Here a novel semi-targeted LC-MS method for analysis of isomeric LPPs, from PC, ChE and TG was developed. Target precursor masses for combinatory inclusion lists of oxidized PC, ChE and TG were predicted using white list of oxidized PUFA units generated by LPPtiger. MS/MS analysis was performed for the selected ions in negative and/or positive ion mode. The fragment ions produced by collision induced dissociation of LPP precursors were further used for data-driven MS3 and/or MS4 analysis yielding structure specific fragment ions necessary to assign type and position of oxidation within PUFA alkyl chain.

The method was validated using *in vitro* oxidized PLs, ChEs and TGs standards prior to analysis of complex biological samples (*in vitro* oxidized plasma and white adipose tissue from obese patients). Thus, using the novel LC-MSn based approach it was possible (i) to detect and identify LPPs in a high-throughput manner, (ii) to reveal their structural diversity in complex biological samples.

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Simultaneous determination of non-polar and polar lipids by serial coupling of HILIC and RPLC to HRMS

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P05 PRIZE

Lipids are a highly diverse group of biomolecules, which fulfill various functions. Hence, many diseases are linked to an altered lipid composition and a comprehensive understanding of the composition is important. Due to the different functions, lipid structures are vastly complex and lipid properties differ greatly. Hydrophilic interaction liquid chromatography (HILIC) enables class-specific separation according to the polar head groups of phospholipids. However, non-polar lipids, such as triacylglycerols, free fatty acids and cholesterol esters, experience insufficient retention. Two-dimensional approaches are used to increase the number of lipid classes. An on-line method was developed that employs HILIC and reversed phase liquid chromatography (RPLC) coupled to high-resolution mass spectrometry (HRMS). Lipids eluting in the void volume of the HILIC column are directed onto an RP column. After the first dimension chromatography is finished, the second dimension chromatography takes place during HILIC equilibration time, resulting in a 50-minute method. This set-up enables the mass spectrometric measurement of both the first and second dimension chromatography, only requiring little additional technical effort. The applicability of the developed method is demonstrated by analysis of a *Saccharomyces cerevisiae* total lipid extract.

Localization of double bond positions by LC-MSMS using online photochemical post-column derivatization

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P06

Double bonds have an influence on lipids chemical, biochemical and biophysical properties. Since 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 presented.

The photochemical Paternò Büchi (PB) reaction was utilized, based on the binding of acetone to the double bonds. Therefore, an additional constant acetone flow and UV-irradiation was used. The acetone adduct was formed due to the reaction of acetone with the double bond resulting in an oxetane. Subsequent MS/MS fragmentation experiments generated diagnostic fragments allowing the localization of double bond positions.

Furthermore, online coupling of the PB approach to HPLC was realized. This chromatographic dimension was added to provide on the one hand additional information on the lipid structures, for instance, concerning constitutional isomers. On the other hand, it enables a recording of spectra with reduced complexity and matrix effects.

Application of the post-column photochemical reaction approach was performed not only by analysis of free fatty acids but also complex lipids like glycerophospholipids. A mixture of constitutional phospholipid isomers, bis(monooleoylglycero)phosphate and dioleoyl-phosphatidylglycerol respectively, was analysed regarding their double bond positions, and lipids from an extract of the green alga *Chlamydomonas reinhardtii* were investigated.

Determination of oxylipins in clinical samples using UHPLC-MS

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P07 **PRIZE**

Oxylipins (eicosanoids, octadecanoids, docosanoids) formed from polyunsaturated fatty acids (PUFA), such as arachidonic acid (AA) and docosahexaenoic acid (DHA) are a special family of lipids. They can be generated by either enzymatic pathways using cyclooxygenase (COX), lipoxygenase (LOX), and cytochrome P450 (CYP450) or non-enzymatic oxidation. Oxylipins play an important role in physiological functions (e.g., cell proliferation, blood pressure, inflammation), and they are associated with diseases (e.g., cancer, diabetes, heart diseases). The most common approach for the analysis of oxylipins in human plasma samples is an ultrahigh-performance liquid chromatography coupling with mass spectrometry (UHPLC/MS) using selected reaction monitoring (SRM) and electrospray ionization in the negative-ion mode (carboxylic group in oxylipin structures). We developed the targeted UHPLC/MS method for determination of 63 oxylipins in human plasma samples, validated (calibration curve, limit of detection, limit of quantitation, matrix effect, recovery rate, precision, and accuracy) and applied to 40 clinical samples (20 breast cancer patient and 20 healthy volunteers). For quantitation, 14 deuterated internal standard were used. In human plasma samples, we detected 35 oxylipins and 20 of them quantified. Results were statistically evaluated using multivariate data analysis (MDA), such as principal component analysis (PCA), orthogonal partial least square-discriminant analysis (OPLS-DA), S-plot, and box-plots.

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Optimization of UHPSFC-ESI-MS and Application Method for Characterization of Biological Samples

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P08 PRIZE

Supercritical fluid chromatography (SFC) is preferably used for analysis of non-polar substance, because the main component of mobile phase is supercritical carbon dioxide. The addition of organic modifiers enhances also the determination of polar compounds. The connection of mass spectrometry (MS) and ultrahigh-performance supercritical fluid chromatography (UHPSFC) with sub-2 μm particles as stationary phase has higher separation efficiency and faster analysis times, which can be applied to lipidomics analysis. Nowadays, the instrumentation includes splitter before the backpressure regulator, which enables employing a make-up solvent for a higher ionization efficiency. Goals were optimization of parameters affecting the sensitivity of nonpolar and polar lipid in short analysis times. Pooled sample was used for the method development and optimization. Lipids were quantified using mixture of internal standards (IS). The increasing of sensitivity by makeup solvents were verified by employing various additives (acetic acid, formic acid, ammonium formate, and ammonium acetate) and flow rates (0.1, 0.25, and 0.5 mL/min). Injection volume and acquisition mode were also investigated. Data were processed by Water´s tools and our laboratory-made software LipidQuant was used for the semi-automated identification, isotopic correction, and quantitation of lipid species. Optimized method was applied to biological samples, and the results were statistically analyzed.

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Development and application of a high resolution mass spectrometry method to identify and quantify faecal lipid species

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P09 **PRIZE**

It is now generally accepted that gastrointestinal system in particular the intestinal microbiome plays an important role in human health and disease. Faecal materials reflect the microbial activity and the analysis of remaining unabsorbed metabolites including lipid species and provide an estimate of metabolic interaction between gut microbiota and host. To identify subtle metabolic variations induced by diet alterations and to characterize the metabolic impact of variations of the gut microbiota, metabolic profiling is becoming increasingly popular. Here we applied flow injection analysis coupled to high resolution mass spectrometry (FIA-HRMS) to identify and quantify lipid species and their abundance in faeces.

Faecal homogenates were subjected to total lipid extraction according to the protocol of Bligh and Dyer (B/D). Analysis of crude lipid extracts was performed by FIA-HRMS. A high heterogeneity was observed in faecal sample from different subjects. However, first experiments showed high amounts of triglycerides and diglycerides in the majority of samples. Species profiles included highly unsaturated species which could be confirmed in MS/MS spectra. Therefore, we are currently validating a FIA-HRMS method for quantification of triglycerides and diglycerides species in human faeces including preanalytical conditions. To get more insight into polar lipid species profile, a three-phase extraction was performed to remove excess neutral lipids by separating neutral and polar lipid classes.

In summary, FIA-HRMS offers a high throughput method to analyse and quantify lipid species profiles of faecal samples. The application of these methods in various samples should provide a comprehensive picture of the faecal lipidome and improve the understanding of the role of the microbiome in human health.

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Phospholipid-mediated ion suppression effects in MALDI mass spectrometry imaging investigated by use of spiked micro-arrays of artificial tissue

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P10 **PRIZE**

MALDI-MS imaging (MALDI-MSI) is increasingly used to visualize the molecular distribution of lipids in biological tissue sections. However, a major hindrance for quantitative (Q)MALDI-MSI applications results from notorious ion suppression effects. The presence of easy to ionize molecular species (e.g. phosphatidylcholine, PC) can dramatically reduce the signal intensities of all other phospholipids (PL). To obtain a deeper insight into these phenomena for PL under MSI conditions, we developed "artificial tissue (AT) substrates that mimic essential characteristics of soft tissue and that can be spiked with controlled concentrations of selected PLs. MS data were acquired with a Synapt G2-S (Waters) in continuous raster mode. For MALDI-MSI, cryosections of the material were coated with matrix using a sublimation/recrystallization protocol.

Best results so far were obtained with a mixture of carboxymethyl cellulose and gelatin as the AT material. To study the PL-derived ion suppression effects in a systematic manner, we used a microarray comprised of 24 PL-spiked slots. In each array the AT was spiked with two different PL classes (e.g., PC, phosphatidylethanolamine (PE), and phosphatidylglycerols (PG)) varying the concentration ratios. MALDI molecular signal intensities of one PL in dependence of a second PL were monitored and compared to those of the respective lipid concentrations. Results show an intricate interplay between PL classes impeding the quantitative analysis of complex biological samples especially in MALDI-MSI applications.

Further experimental parameters that can influence ion suppression and which were investigated to some effect concern the choice of MALDI matrix, polarity of the ion detection mode, influence of the MALDI laser wavelength, laser fluence and focal spot size. Finally, we present data on ion suppression in laser-induced postionization (MALDI2), investigated using an exemplary PC/ PG mixture.

Fractionation of the 13C-labeled yeast lipidome by prep-SFC for a new scope of internal standardization

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P11 PRIZE

It is commonly accepted that internal standardization (ISTD) is an absolute prerequisite for accurate quantification in lipidomics. In this context, our group recently introduced a novel 13C-standard production pipeline which we coined as lipidome isotope labeling of yeast (LILY). The use of – this fast and efficient in vivo 13C-labeling strategy in Pichia pastoris – offers more than 200 13C-labeled lipids for internal standardization from classes such as Cer, TG, HexCer, PC, DG, PE,PA, LPC, PG, PI, Hex2Cer, ST, PS, Co, LPE. In order to reduce the complexity while increasing its versatility, we developed a strategy for class specific fractionation. More specifically, the yeast lipidome was fractionated in its lipid classes by preparative supercritical fluid chromatography (SFC). The method with the attribute of green technology benefits proved to be highly efficient over other investigated preparative methods for lipid class separation.

The fractionated and enriched 13C-labeled lipidome not only helps to further investigate the yeast lipidome itself, it can also be better adapted to samples of interest or used for more specific applications than a global lipidomics approach. The quantitative results of the fractions and preliminary results of the applications of this new more flexible ISTD will be shown.

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Are lipid oxidation processes the key for effectivity of cold physical plasmas?

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P12

Biological membranes are important interfaces in living systems forming highly selective barriers. In addition, biomembranes are one of the first interaction sites of external compounds, including reactive species (RxS, x = O, N, S). Transient or permanent changes in membrane mechanics and/or oxidation state relay signals into the cellular interior that can be exploited to control cell function. An emerging source for the spatially controlled delivery of reactive species is cold physical plasmas which are under investigation regarding their clinical potential in chronic wound management and cancer therapy (1,2). So far, the exact biochemical mechanisms beyond are underexplored.

Lipids, i.e. phospholipids, can be targeted by plasma-derived species in liquids systems (3). Here, we describe the investigation of gold supported POPC lipid bilayer by cold plasma treatment using electrochemical approaches (cyclic voltammetry, differential pulse voltammetry), atomic force microscopy, and high-resolution mass spectrometry. Depending on the plasma parameters and treatment time, oxidative changes of membrane integrity and chemical composition was observed. Cleavage reactions at the lipids head group and side chain oxidation, predominantly at Δ^9, were found. The responsible reactive species were determined to be OH radicals, atomic oxygen, and singlet oxygen. Interestingly, no clear role could be attributed to reactive nitrogen species.

In conclusion, a role of (advanced) lipid oxidation products and changes to membrane fluidity can be assumed for cold plasma initiated cellular processes. Native lipid composition influences the outcome of RxS attack, suggesting a partial selectivity of plasma treatment.

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Chasing for artefacts in MALDI-MSI - Use of HILIC-based nano-HPLC-ESI MS for phospholipid quantification in (micro-dissected) tissue sections

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P13 PRIZE

Phospholipids (PL) exhibit numerous essential physiological functions. For example, they are the main structural elements of cell membranes and certain PLs serve as signaling molecules. This multitude of functions comes with a high structural diversity and sizable differences in expression levels. Therefore, detailed knowledge about the PL composition in tissues can provide valuable information about certain states of disease. This is one reason, why quantitative determination of the lipidome by dedicated MS approaches finds a tremendous interest in the biomedical community. A multitude of methods for this purpose is established based on shotgun or LC-MS set-ups, usually requiring larger quantities of material. MALDI-MSI imaging on the other hand can provide qualitative information about the distribution of PLs in thin tissue sections. In this method however, quantitation is severely hampered by ion suppression effects that may differ greatly between regions of the sample. In the presented approach we combine and compare (Q-)MALDI-MSI with nano-HPLC-ESI-MS of extracts collected from the same sample. Based on a MALDI-MSI measurements of a mouse brain sections, regions of interest, that show a homogenous distribution of lipid signals, were identified, excised, and collected by laser micro dissection. Afterwards the PLs were extracted and quantitated by nano-LC-ESI-MS. An Ultimate 3000 (Dionex) was used as HPLC system and coupled to a MALDI/ESI injector (Spectroglyph) mounted on a QExactive Plus (Thermo Fisher). With a DIOL-HILIC column as stationary phase most major PLs classes are efficiently fractionated, while the high mass resolving power and MS/MS capability of the orbitrap ensures differentiation of PLs according to their overall alkyl chain composition. Because ion suppression is mainly mediated by the PL head groups, the separation by PL class reduces the number of required exogenous PL standards needed for quantification. Comparing the quantitative results of the regions of interest and MALDI-MSI images reveals major mismatches between the MSI signal intensity distribution and the actual underlying molar content. This is presumable caused by regionally specific ion suppression effects. The presented results therefore highlight the need for robust methods for normalization and quantification in MALDI-MSI of lipids.

Systems biology of the unfolded protein response

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P14 **PRIZE**

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. 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 significantly enriched or depleted. 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 modelling and integration with the results that obtained in the earlier modelling approaches in regard to the ER stress - and unfolded protein response.

MS- and NMR-based structural analysis of lipoteichoic acids isolated from three Streptococcus suis serotype 2 strains

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P15

Crucial interactions of bacteria with their hosts are mediated by various bacterial cell wall components. Besides peptidoglycan and lipoproteins, wall teichoic and lipoteichoic acids (LTA) are the major constituents of Gram-positive cell walls. LTA are non-covalently anchored in the cell membrane via a diacylglycerol-containing glycolipid anchor, which is in turn substituted with a poly-alditolphosphate chain (containing either glycerol-phosphate (GroP) or ribitol-phosphate) as backbone. The nature of these two structural parts is highly varying between Gram-positive species. *Streptococcus suis* serotype 2 is an important porcine and human pathogen and its LTA has been suggested to contribute to its virulence. Here, using high resolution NMR spectroscopy and MS analyses, we characterized the LTA structures from three *S. suis* serotype 2 strains differing in virulence, sequence type (ST), and geographical origin. Our analyses revealed that these strains possess - in addition to the typical poly-GroP-containing type I LTA present in other streptococci - a second, mixed-type series of LTA molecules of high complexity. We observed a ST-specific difference in the incorporation of glycosyl residues into these mixed-type LTAs. We found that strains P1/7 (ST1, high virulence) and SC84 (ST7, very high virulence) can attach an 1,2-linked alpha-D-Glc*p* residue as branching substituent to an alpha-D-Glc*p* that is 1,3-linked to glycerol phosphate moieties and that is not present in strain 89-1591 (ST25, intermediate virulence). By detailed tandem mass spectrometric analyses we could show the consecutive order of the two different repeating unit types in these LTA molecules. In *S. suis* strains P1/7 and SC84, a defined subset of LTA molecules with 3-10 GroP repeats was found to be elongated with 1-10 repeats of the more complex glycosyl residue-containing units. To the best of our knowledge, this is the first example of such a regulated synthesis of a mixed-type LTA. In contrast, strain 89-1591 could glycosylate its LTA at the glycerol *O*-2 position, which was not observed in the other two strains.

Lipid Profiles as Marker for Antimycobacterial Treatment

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P16

Tuberculosis (TB) is caused by *Mycobacterium tuberculosis* (*Mtb*) and mostly affects the lungs. According to the WHO, a total of 10.4 million people developed active TB in 2016. Because of the worldwide increase in multidrug resistant (MDR) TB, treatment regimens become more complicated and may last a prolonged period of time of up to 24 months. Conventional methods to determine treatment efficacy in patients include the determination of sputum conversion of mycobacterial growth in liquid or solid. These methods mostly depend on the metabolic state of the bacillary population under treatment and are very labor- and time-intensive. Until now, there are no biomarkers available that reflect the longitudinal decline and eventually eradication of *Mtb* during therapy of TB. In light of the increasing incidence of MDR TB there is, however, an urgent need for an analytical highly sensitive and easy to use approach to determine a biochemical correlate of a longitudinal treatment success.

In this study, we analyzed in *Mtb*-infected interleukin-13-overexpressing mice which greatly reflect the pathogenesis in TB patients, how bacterial load and antibiotic treatment influence the lipidome of the lung. Lipids from infected mice were extracted by the methyl *tert*-butyl ether (MTBE) protocol at different time points during antibiotic treatment. Subsequently, shotgun lipid*omics* was performed with nanoESI-MS/MS using a Q Excative Plus coupled with Triversa Nanomate and LipidXplorer was used for lipid identification and quantitation.

Our analysis indicates that the tuberculostearic acid containing phosphatidylinositol PI (16:0/19:0) provides sufficient specificity and sensitivity to determine *Mtb* burden in correlation to the bacterial load determined by conventional methods. Furthermore, we present *Mtb* related imprints in the lung lipidome of the host and show first results for shotgun lipid*omics* in a mouse model.

LC-MS-MS approach for investigation of cyclooxygenase-2 expression

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P17 **PRIZE**

Eicosanoids and other oxylipins function as important lipid mediators and play a major role in regulating many physiological functions such as intracellular signaling, inflammation and pain. They are formed in the arachidonic acid (ARA) cascade via three enzymatic pathways: cyclooxygenase (COX), lipoxygenase (LOX) and cytochrome P450 (CYP).

The investigation of the ARA cascade is mainly done by direct quantification of oxylipins via LC-MS/MS. However, with this approach only little information on molecular mechanisms responsible for changes in the oxylipin pattern are gained. Quantification of enzyme expression levels is additionally required, as e.g. reduced oxylipin levels may result from direct enzyme inhibition or decreased expression.

In the work presented here, an LC-MS/MS method was developed for targeted analysis of COX-2 enzyme expression, allowing comprehensive monitoring of the COX-2 branch of the ARA cascade together with an established targeted oxylipin method. Unique peptides ("proteotypic peptides, PTP) arising from tryptic digestion of the target enzyme were selected based on data from *in silico* digestion. Sites of natural variants, e.g. nonsynonymous single nucleotide polymorphisms (nsSNPs) and post-translational modifications were avoided by considering data from different proteomics platforms. Criteria for selection of PTP also included a high predicted cleavage probability as well as the absence of chemically unstable residues such as tryptophan or asparagine, as far as possible.

Utilizing a denaturing lysis buffer and sonication, the protein fraction was extracted from cells and treated with a chaotropic reagent, mediating unfolding of the proteins. Intramolecular disulfide bridges were reduced and subsequently alkylated with iodacetamide (IAA) before tryptic digestion. After chromatographic separation of the desalted peptides, they were measured on a QTRAP instrument following positive ionization in scheduled multiple reaction monitoring (sMRM) mode, enabling sensitive detection in the ng/mL range. Three selected peptides were optimized and the method was applied for comparison of COX-2 expression in different human cell lines.

Characterization of the human plasma lipidome using LC-IM-qTOF-MS

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P18 **PRIZE**

There are at least two big challenges in analysing lipids. On one hand, there are about 100,000 lipids estimated to occur in nature but only 40,000 lipids are reliable identified and reported in the biggest database (LipidMaps). On the other hand, there is a large number of isobaric lipids, which are difficult to separate by liquid chromatography. [1] Ion mobility spectrometry (IMS) is an additional separation dimension, which allows the separation of coeluting isobaric compounds in the gas phase according to their size-to-charge ratio leading to an increased separation power. Furthermore, the collision cross section can be easily determined using a drift-time IMS, which is useful as an additional parameter for the identification of lipids in complex biological samples such as human plasma.

A three-dimensional lipidomics approach with a high separation power was developed by using liquid chromatography coupled to the Agilent 6560 IM-qTOF-MS. For the chromatographic separation a long 60 minutes gradient using a mixture of water/acetonitrile and acetonitrile/isopropanol with a C18 column was used. In addition, MS parameters were optimized in both positive and negative ionization mode to increase the coverage of the detected lipid species and the sensitivity allowing the detection of low abundant lipids.

Limits of detection (LOD) and quantification (LOQ) of 12 different lipid classes were determined in human plasma using deuterated standards. The described method shows low LODs below 100 nM (2 pmol on column) for most lipid classes. It ranged from 3 nM for the phosphatidylethanolamines to 7000 nM for cholesterol esters. Additionally, the accuracy was determined using matrix-match calibration. For the majority of the investigated lipid classes the accuracy was higher than 80%, except for triglycerides and sphingomyelins (64% and 78%, respectively). The method was applied to characterize the human plasma lipidome. In total we found about 3,000 lipid features, of which roughly 1,000 lipids were identified on species level using an inhouse build database.

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Cold induced eicosanoid signature differs between BAT positive and BAT negative subjects

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P19 PRIZE

Introduction

Obesity is a consequence of energy dysbalance when energy intake exceeds energy expenditure. Brown adipose tissue (BAT) in contrast to white adipose tissue dissipates chemical energy in form of heat through a process called uncoupled respiration. The strongest known activator of BAT *in vivo* is cold exposure which induces uncoupling protein-1, a key thermogenic factor. Moderate cold exposure has been demonstrated to increase the uptake of fludeoxyglucose (^{18}F) in human BAT depots which can be visualized by positron emission tomography in combination with computer tomography. Cold-induced BAT activity is associated with enhanced energy expenditure, so-called cold-induced thermogenesis (CIT). This study aims to investigate the effects of short term cold exposure on BAT activity, CIT and circulating lipid mediators in human plasma samples of healthy BAT positive as well as BAT negative subjects.

Methods

For the sample preparation, proteins were precipitated and the lipid fraction was purified and concentrated using solid phase extraction. An untargeted reverse phase LC-MS approach with an UHPLC instrument (Thermo Fisher Scientific™ Vanquish™) hyphenated to an HR orbitrap mass spectrometer (Thermo Fisher Scientific™ Q Exactive™ HF) was conducted to obtain relevant features. Data evaluation was performed with the commercial software TraceFinder™ (Thermo Fisher Scientific™).

Results and Discussion

60 identified free fatty acids including classic pro-inflammatory eicosanoids such as PGE2, as well as pro-resolving mediators like RvE1 were investigated. Furthermore, a set of 145 features potentially representing eicosanoid-like molecules were detected and could be correlated to BAT positive subjects as well as to cold-induced thermogenesis suggesting possible biomarkers.

Analysis of Fatty Acids and Oxylipins in Platelets by Combined Targeted and Untargeted LC-MS2 Method

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P20 PRIZE

Platelets are essential components in blood and their main role is blood clotting in case of injury. On the biochemical level platelet activation induces cascade changes in lipid compositions and concentrations, respectively – non-active lipids are converted by enzymes into bioactive compounds: for example fatty acids, thromboxanes and prostaglandins. One of the significant activation pathways is the conversion of prostaglandin H2 (PGH2) into thromboxane A2 (TXA2). In this reaction the equimolar amount of 12S-hydroxyl-5Z,8E,10E-heptadecatrienoic acid (HHT) is produced, together with TXA2, an effective autocrine platelet agonist. However, TXA2 is highly unstable and is rapidly converted to thromboxane B2 (TXB2) and as such might by analyzed. HHT is stable, but it can be oxidized to 12-oxo-5Z,8E,10E-heptadecatrienoic acid (KHT). The biological function of KHT is still unknown.

The goal of this project was to obtain a sensitive method for analysis of fatty acids and their derivatives in platelet extracts. The new targeted/untargeted (Qual/Quant) UHPLC-ESI-QTOF-MS/MS method targeted three compounds to accurately analyze them quantitatively: TXB2, HHT and KHT. Data independent acquisition with SWATH (Sequential Window Acquisition of all Theoretical Fragment Ion Mass Spectra) was utilized to analyze compounds with precursor m/z 50 to 500. The window sizes and accumulation times were variable, because better sensitivity and selectivity of targeted compounds were obtained when their windows were narrower and with longer accumulation time.

The newly developed LC-MS method was used to analyze 22 samples from 11 donors. The samples were divided to 2 groups: platelets activated by thrombin and resting platelets. The analysis shows that concentrations of targeted compounds were significantly different between the groups.

Comparison of monophasic isopropanol:water mixtures versus classical biphasic extraction protocols for comprehensive UHPLC-MS-MS lipidomics analysis of Hela cells

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P21 PRIZE

Lipidomics allows wide characterization of lipids in biological samples, and because of that the development of workflows which can be as comprehensive, reproducible, fast and simple as possible must be encouraged.

In this study four different extraction protocols were compared in order to evaluate their performance for lipidomics studies in Hela cells. Tested extraction protocols were 1) methanol/methyl-tert-butyl ether/H_2O (Matyash method), 2) methanol/chloroform/H_2O (Bligh and Dyer method), 3) isopropanol/H_2O 75:25 v/v (IPA 75) and 4) isopropanol/H_2O 90:10 v/v (IPA 90).

Data were acquired using reversed phase UHPLC coupled to high-resolution QTOF-MS instrument in positive and negative ion modes, using data independent acquisition (DIA) with sequential window acquisition of all theoretical fragment ion spectra (SWATH). This allows acquiring MS and MS/MS data comprehensively over the entire chromatogram and across all samples. This makes possible to extract MS/MS chromatograms as well. Data were processed by using MS-Dial software for peak finding, identification and alignment of features. Comparison of extraction protocols was based on the coefficient of variation for intensities of detected features, the extraction recovery of isotopically labeled internal standards, recovery of endogenous lipids, the diversity of lipid classes that can be extracted, the amount of extracted lipids and the complexity of each protocol.

The results demonstrated that IPA 90 extraction provides similar performance to Matyash protocol and better than Bligh and Dyer protocol. On the other hand, it is less complex because it does not require a tedious phase separation or the use of glassware, which converts this protocol in to an excellent alternative for performing lipidomics studies.

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Software Utilizing Positive and Negative Ion MS2 MS3 HCD and CID Spectra for Improved MSn Lipid Identification

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P22

The application of lipidomic profiling to disease phenotype analysis is a rapidly growing aspect of translational medical research. Identification of lipids by untargeted lipidomics requires sophisticated software with an extensive lipid database containing the product ions and neutral losses associated with each lipid adduct ion. We present here the data processing of bovine liver and heart total lipid extracts (Avanti Polar Lipids) using the latest version of Thermo Scientific LipidSearch software. New algorithms were introduced specifically to reduce false positives, improve quantitation and to automate searching of MS^n data obtained by higher collisional energy (HCD) and linear ion trap collisional induced dissociation (CID) fragmentation methods. MS^2 and MS^3 spectra were searched against the predicted product ions and neutral losses for all of the potential lipid species within ± 5 ppm precursor and product ion mass tolerances. Each potential lipid identification was ranked by mass tolerance, match to the predicted fragmentation and the fraction of total MS-MS intensity for those predicted fragment ions. Positive and negative ion modes data was aligned within a chromatographic time window and merged into a single lipid annotation in the results table, providing higher confidence in lipid identifications. The alignment results were then filtered by minimum number of data points, signal-to-noise ratio, main adduct ion, match score, ID quality, and coefficient of variation from replicate sample injections. Compared to the results generated only from dd-MS^2 HCD results, the combination of HCD and CID MS^2/MS^3 gave significantly higher quality lipid identifications in the same analysis time.

Robust and Sensitive LC-MSMS Based Plasma Lipid Profiling on a Thermo Scientific Q Exactive HF-X Mass Spectrometer

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P23

Lipid profiling can be compromised by their extraction from biological samples, chromatographic separation, ionization and detection by mass spectrometry. The Thermo Scientific Q Exactive HF-X has an increased scan speed and sensitivity which should both benefit lipid profiling experiments. In this study, we optimized a complete workflow for plasma lipidomics with improved sensitivity and quantitative performances.

Human plasma samples were spiked with known concentrations of SPLASH Standard (Avanti) and extracted with varying sample amounts. Lipids were separated on a 15 cm Accucore C30 column and analyzed on the Q Exactive HF-X. Several instrument parameters were evaluated, including the detection mass range, ion transmission and data dependent parameters. LipidSearch software was used for identification and quantification of molecular lipid species.

An evaluation of the optimum plasma volume was conducted by extracting the ion signals for 4 known plasma lipids ranging in abundance from low (Cer (34:1;2) and Cer (38:1;2)) to high (PC (34:2)). Replicate extractions confirmed the reproducibility of the method with coefficient of variations below 25% for all standards. Further optimizations included the limitation of in-source fragmentation of labile lipids and improvement of MS^1 detection across the studied lipid classes. Calibration curves were generated to determine the linearity of detection and limits of quantification. Finally, data dependent parameters were evaluated in order to maximize the number of high quality MS^2 spectra generated and identified lipids. As a results, the optimized method resulted in the confident identification and quantification of more than 500 lipids from a human plasma sample.

Coactosin-like 1 is a novel regulator of thrombosis and hemostasis

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P24 PRIZE

Background: Cytoskeletal rearrangements play an important role in platelet activation by facilitating platelet shape change and the formation of filo- and lamellipodia that are critical for normal hemostasis. Coactosin-like 1 (Cotl1) is a small filamentous actin-binding protein that consists of a single actin-depolymerizing factor homology (ADF-H) domain. Cotl1 was shown to prevent cofilin-mediated depolymerization of actin filaments and to regulate the activity of 5-lipoxygenase that is implicated in leukotriene synthesis.

Aims: We investigated the role of Cotl1 in platelet production and function *in vitro* and *in vivo*.

Methods: We generated the first MK-/platelet-specific Cotl1-deficient ($Cotl1^{fl/fl\text{-}Pf4Cre}$) mouse line. Platelet morphology and function was assessed *in vitro* under static and dynamic conditions, as well as *in vivo*.

Results: $Cotl1^{fl/fl\text{-}Pf4Cre}$ mice displayed unaltered platelet counts and size compared with controls, suggesting unaltered thrombopoiesis. In addition, platelet activation and aggregation responses to various platelet agonists were normal in Cotl1-deficient mice. Surprisingly, despite the proposed inhibitory role of Cotl1 in actin dynamics, actin polymerization and cytoskeletal organization were unaltered in platelets lacking Cotl1 compared with controls. Strikingly, however, $Cotl1^{fl/fl\text{-}Pf4Cre}$ platelets showed a significantly reduced adhesion and aggregate formation on collagen I in a flow adhesion assay indicating impaired cellular activation under conditions of shear flow. *In vivo* this defect translated into slightly prolonged bleeding times and a profound protection from occlusive arterial thrombus formation. A combination of altered GPIb-vWF interaction, a reduced platelet stability and a decreased leukotriene production might account for the observed thrombus formation defect of $Cotl1^{fl/fl\text{-}Pf4Cre}$ platelets, which is under current investigation.

Conclusions: In summary, we show for the first time that Cotl1 is dispensable for actin dynamics in platelets, but we identified a central regulatory role of Cotl1 in hemostasis and arterial thrombosis.

Keywords: Cotl1, cytoskeleton, platelets, thrombosis and hemostasis

Is the porcine lung lipidome a valid model for the human lung lipid metabolism

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P25 PRIZE

The integrity of alveolar structures are essential for normal lung function. In respiratory diseases like Chronic Obstructive Pulmonary Disease (COPD) dramatic perturbation in the fine structure of alveoli are observed, which are reflected in the lipidome. In a first proof of concept study we showed that the lipid metabolism of the human lung is influenced by various traits like age, sex and BMI [1]. Lung tissue samples of healthy individuals are practically not available for ethical reasons, consequently, residual cancer-free lung tissue sections were histologically evaluated and further processed for shotgun lipidomics. This sampling strategy for the clinical cohort results in bias towards older individuals, increased number of smokers as well as higher portion of male participants. At the same time genetic background and different lifestyle parameters like nutrition and smoking are unsupervised in this recruitment strategy.

In this context we searched for alternative animal models that will help to control for such factors. We used domesticated pigs which have a high similarity to humans in general anatomy and histology of the lung and physiology. Pig models are already used to study lung diseases like cystic fibrosis, COPD [2], lung inflammation and lung hyperinflation. Pulmonary surfactant of porcine lungs is even used as therapeutics for the respiratory distress syndrome in premature infants.

For this study we analyzed lipid profiles of 66 lung tissue samples of female, male and neutered male pig breeds like the "Angeln Saddleback (Angler Sattelschwein)", "German Large White (Deutsches Edelschwein)", "German Landrace (Deutsche Landrasse)" or crossbreeds like "Pietrain x German Large" and scored corresponding histological sections. First histological investigations showed increased levels of inflammation in the lung in almost all samples, as well as unusually enlarged alveolar size. Whether these changes in the lung are related to the pig breed or systemic effects due to rearing conditions, are part of the investigation.

Shotgun lipidomics approach was applied to profile the lung lipidome using a Q Exactive Plus (Thermo, Bremen, Germany) coupled with Triversa Nanomate (Advion, Ithaka, USA) to automatize sampling and as nano-ESI source as reported earlier. We quantified approximately 400 lipids from 14 lipid classes including PC, PC-O, PE, PE-p, PS, PI, PG, CE, SM, Cer, HexCer, CL, DAG, and TAG.

We will discuss the outcome of the systematic lipidome comparisons using correlation analyses as well as the LUX score approach to measure lipidome homology.

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An Improved Web Based LUX Score Browser for Lipidomes Homology

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Recent research has shown that the cellular content of lipids and its localization within compartments are essential for specific cell functions. Therefore, the cellular lipidome can be perceived as signatures for cell identity, disease status and developmental stage. In the last decades, technological advances regarding lipid identification and quantification allowed to gain insight into the vast complexity of cellular lipidomes.

Recently, we introduced the LUX score as a metric to determine lipidome homologies [1]. This approach enables systematic lipidome comparisons that take into account lipids structural diversity.

The original programmatic implementation was a command line application running exclusively on Linux. Several problems were identified limiting usability of the approach (a) fragmented implementation using several PERL scripts (b) long computation times, which did not allow processing of complex lipidomes, (c) a restricted SMILES generation process, and (d) no direct incorporation of error modelling functionality.

Therefore, we released the LUX score Browser V.1.0 which is the desktop version aiming at overcoming the limitations of the original implementation. Moreover, we upgraded the desktop version into a web application considering different levels of improvements. The LUX Score web application is now based on the Java-based Spring Boot framework to implement a model-view-controller design. The view is provided via Thymeleaf templates and uses jQuery for enhanced features and interactivity. The application is deployed on an embedded Apache Tomcat servlet container. The execution of LUX Score is handled asynchronously and the result access and the progress monitoring are provided via a unique session URL. We use Docker Swarm and stack deployment to provision the LUX score application on the server side using traefik as a load-balancing reverse proxy to control data exchange.

As a result, the web version of the LUX score browser offers the following advantages, (a) improved user friendliness because only uploading of input data is required, (b) fast and accurate SMILES generation with respect to isomers space and identification accuracy, (c) integration of error modelling functionality, (d) platform independence and (e) user support via the LIFS online portal. The web based LUX score application generates robust SMILES structures that have a unified and formal chemical space that can be used to calculate lipidomes homology in a more efficient way. Thus, the results of the LUX score metric can be generated easier, faster and show improved results compared to the original LUX score metric. As a benchmark test, we used the published yeast lipidome data set that contains 8 lipidomes consisting of 248 unique lipid species and the *Drosophila* lipidome comprising of 12 lipidomes and 356 unique lipid species. We could shorten the processing time by a factor of 4 for the *Yeast* lipidome data and by a factor of 20 for the *Drosophila* lipidome comparison. Results of LUX score based lipidome comparison using the new SMILES generator sufficiently distinguish six *Drosophila* larval tissues (gut, brain, wing disc, salivary glands, fat bodies and lipoprotein) either grown on yeast or protein enriched food. Further improvements could be achieved for clustering of yeast strains lipidomes according to growth temperature and mutation in either Elo1, Elo2 or Elo3 fatty acid elongases.

In our future work, we will implement machine learning algorithms to incorporate lipid quantities and integrate phenotypic data for lipidome homology computation. For better user interaction we will offer an online input data validator to inform users about any problems concerning the input format.

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Lipid mediator quantitation and lipidome analysis of human neutrophils infected by M. tuberculosis

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Lipid mediators (LM) play a crucial role during microbial infection and mark the inflammation status of the host. To develop "Host directed Therapies that favorably influence the inflammatory response during antibiotics therapy of *M.tuberculosis* (*Mtb*), we monitored LM quantities and lipidome perturbations of *Mtb* infected neutrophils.

Generally, the identification and quantification of PUFAs and their derivatives are analyzed using liquid chromatography - tandem mass spectrometry (LC-MS^2). However, for general lipidome characterization we utilize an established shotgun approach. Here, we systematically tested if efficiency of data acquisition and data quality would allow to perform lipidome profiling and LM quantitation from one extract and one LC-MS^2 acquisition.

Recently, we developed a parallel reaction monitoring (PRM) based LC-MS^2 approach to study the LM profiles using a SPE-free extraction procedure [1]. In our first approach we test the efficiency of the LC-MS approach to study LM and lipidome profile on a cell culture based infection model. For this investigation, freshly isolated human polymorphonuclear leukocytes (PMN) have been divided in a control and infected group, respectively. After an incubation time of 3 h at 37 °C, the release of LMs have been induced by adding LPS to the media for 30 min. Finally, the incubation was stopped directly by cooling on ice and the reaction solution have been transferred in a ratio of 1:5 in a tube with methanol and butyl-hydroxy-toluol to stop metabolic activity and kill *Mtb*.

We were able to quantify 8 LMs from total lipid extracts using LC-MS^2 with PRM using a Q Exactive Plus (Thermo, Bremen, Germany). For 15 HETE and 12 HETE increased in levels were detected in the medium of *Mtb* infected cells when compared to the control group. In the next step we used the MS^1 information acquired at specific retention time windows to identify lipid species. In this manner, we were able to quantify 56 lipids applying LipidXplorer. Moreover, the evaluation of the data exhibited changes in the lipid profile of LPE between the non-infected and the infected neutrophils.

As benchmark we compared shotgun lipid*omics* with the lipid extract. To study the lipidome of the PMNs the lipid extract have been prior diluted in a MS-Mix ($CHCl_3$:MeOH:iPO, v/v/v 1:2:4 with 2.9 mM NH_4AC) 1:10. The infusion of the sample to the MS have been made applying TriVersa NanoMate (Advion, Ithaca, USA).

By applying LipidXplorer on the acquired data we were able to identify 215 lipid species in 15 different phospholipid-classes like PC.0, PE, PI, PS, LPC, LPE, SM, CER, TAG, DAG, MAG. With the shotgun approach, we were able to detect the same changes in the content of LPE and additionally in PS in comparison of infected neutrophils to the non-infected.

Our first investigation showed that LC-MS^1 data collected during LM analysis enabled to profile a portion of lipids of the generic lipid*omics* screens. We think that with further improvements in data analysis we can have an in-house validation for shotgun lipid*omics* based profiles integrated in LM driven studies.

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Proteomic analysis of functional processes and metabolic pathways during adipogenesis by establishing SRM-methods

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P28 PRIZE

Adipogenesis describes the process of differentiation of a mesenchymal stem cell into a matured adipocyte. Thereby the adipose tissue is formed, which primary function is to store chemical energy in form of triglycerides and cholesterol esters and vice versa the lipolysis, the process that releases the stored energy in form of fatty acids and glycerol. As a consequence, adipocytes are key players in the bodys energy homeostasis [1]. Dysfunctions of adipocytes and the formed adipose tissue have severe consequences resulting in metabolic and cardiovascular diseases [2, 3].

The aim of our work was the global proteomic analysis and characterization of functional processes and metabolic pathways of primary stem cells during adipogenesis. In addition absolute quantification of key players of central metabolic pathways was performed and an in house build database **STAMPS** was created to generate targeted SRM methods.

To achieve those goals, we first generated samples covering different time points during the adipogenic process of OP9 cells treated with a specific adipogenesis inducing cocktail. By performing a subcellular fractionation of the cells generating cytosol, nucleus, membrane and histone fractions, we were able to cover the spatial and time dependent dynamics of over 6300 proteins in a global approach using nano LC-MS/MS. Furthermore, 7 metabolic pathways, involved in carbon, energy and fatty acid metabolism, as well as 2 signaling pathways were investigated with label free and SRM based methods. In this process, key proteins were validated and absolutely quantified using isotope labeled standard peptides.

In conclusion the results of this combinatorial approach of a global and a targeted mass spectrometry strategy show the dynamic regulation of entire metabolic pathways as well as single proteins during the time course of adipogenesis. To further complete the understanding of this complex mechanism subsequent objectives include the correlation of the proteomic data with metabolomic and lipidomic datasets by using SIMPLEX [4]. This will enable us to shed light even into the darkest corners of the adipogenesis.

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Plasma lipid environment in hyperlipidemic and hypercholesterolemic mice enhances platelet and coagulation activation independent of age

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Rationale: Hyperlipidemia and hypercholesterolemia as well as aging are well-established risk factors for cardiovascular diseases (CVD). However, it is unclear how differences in plasma lipid composition can affect a prothrombotic phenotype.

Objective: To investigate how elevation of plasma lipids, modified by genetic modification and age, influence platelet and coagulation tendency. Mice deficient in apolipoprotein E (ApoE) or low-density lipoprotein receptor (LDLR) were used as models for hyperlipidemia and hypercholesterolemia, respectively.

Methods and Results: Plasma cholesterol levels were higher in (aged) *Apoe*$^{-/-}$ mice than in *Ldlr*$^{-/-}$ mice. Thrombus formation *ex vivo* was investigated in blood from young and aged *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ versus wild-type (WT) mice by perfusion over microspots of thrombogenic surfaces. Parameters of coagulant thrombus formation (with tissue factor) over time were increased for both *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice as compared to WT mice, independent of age. Parameters of non-coagulant thrombus formation on collagen under flow showed a pronounced gain-of- platelet-function in *Ldlr*$^{-/-}$ mice as well as in *Apoe*$^{-/-}$ mice when compared to WT, again independent of age. Interestingly, proteomes of *Ldlr*$^{-/-}$ and *Apoe*$^{-/-}$ platelets were largely comparable to that of WT platelets, although minor differences were observed. Platelet lipidomic profiles indicated increases in both cholesterol levels and glycosphingolipids in platelets from both knock-out mice. Transplantation of WT or *Ldlr*$^{-/-}$ bone marrow into *Ldlr*$^{-/-}$ recipient mice resulted in similarly, high gain-of-platelet-function. Hence, these platelets appeared to acquire this prothrombotic phenotype from circulating in the hyperlipidemic environment.

Conclusions: The prothrombotic propensity of *Apoe*$^{-/-}$ and *Ldlr*$^{-/-}$ mice appears to be age-independent, and driven by a gain-of-function of platelet and coagulant activity. It appears that the platelets adapt to the altered plasma environment, which is mostly reflected by the platelet lipid profile.

Metabolic Differences of Atherosclerosis Pathogenesis with a Focus on Pseudoxanthoma Elasticum (PXE) in both Human and Mice

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P30 PRIZE

Introduction

Atherosclerosis is a chronic inflammatory disease and a leading cause of death worldwide. It begins with an accumulation of lipid laden foam cells in the intima of arteries, which then progress to intimal fibroatheroms [1]. It is known that young females have a lower incidence of atherosclerosis; however, after menopause the risk increases and may even exceed that seen in males [2]. PXE is a metabolic disorder caused by mutations in the ABCC6 gene which lead to accumulation of mineralized and fragmented elastic fibers that primarily affect skin, eyes, cardiovascular system and thereby premature atherosclerosis [3]. Additionally, metabolic differences between male and female PXE-diseased patients can be recognized. In order to investigate the metabolic distribution in patients with atherosclerosis and PXE, as well as in an ABCC6 mouse model, MALDI mass spectrometry imaging (MSI) is a novel tool.

Methods

Fresh biopsy tissue samples of healthy and PXE diseased human skin were rapidly frozen in liquid N2, cut into thin sections and mounted onto Iridium Tin Oxide slides. 2,5-Dihydroxybenzoic acid (DHB) was sprayed onto the sections by a TM-Sprayer (HTX Technologies, LLC). The sections were analysed by MALDI-TOF-MSI (ultrafleXtrem; Bruker). Additionally, vibrissae and kidney of ABCC6 / and wild type mice were analysed in the same way. Data exploration and cluster analysis were performed using FlexImaging (Bruker) and the R-package Cardinal [4]. Histological staining (Haematoxylin and Eosin, Alizarin red) were used to identify calcifications in PXE diseased tissue.

Results and Discussion

Initially we analysed atherosclerotic arteries with MALDI-MSI at a resolution of 20 µm. We localised known biomarkers like lysophosphatidylcholine (496.3 m/z) with a clear accumulation in the centre of fibroatheroms. Furthermore we identified 30 characteristic metabolites for adventitia (e.g. 326.4 m/z), media (e.g. 566.3 m/z) and intima (e.g. 685.6 m/z) each. We were able to visualise differences between healthy and PXE-diseased mouse vibrissae and kidneys, and human skin using MALDI-MSI. These differences were confirmed by Spatial Shrunken Cetroid Clustering [4] of the mass spectrometry data.

Conclusion

MALDI-MSI proved to be a useful and valid tool to investigate the inflammatory processes and calcification in atherosclerotic fibroatheroms. Using the R-package Cardinal we were able to efficiently process large MSI data sets and perform cluster analysis. In future works we hope to visualise the already known sex differences in atherosclerosis and PXE with MALDI-MSI.

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SIMPLEX: from extraction to underlying molecular mechanisms in cardiomyopathy

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P31 PRIZE

Despite significant therapeutic advances, there is no unified, complete mechanism explaining how exactly various cardiomyopathies biochemically manifest and progress nor explaining why this cardiac conditions result in heart failure. "Caveolinopathies are caused by mutations in the sarcolemmal protein Caveolin-3 (CAV3) and can manifest as myopathic diseases often associated with cardiomyopathy. To gain deeper insights into the underlying molecular mechanisms, a comprehensive and representative analysis demands a deep and parallel coverage of a broad spectrum of molecular species. Therefore, the previously established SIMPLEX procedure (SImultaneous Metabolite, Protein, Lipid EXtraction) (1) was applied to examine the molecular basis of an inherited dilative cardiomyopathy from a transgenic mouse model carrying a dominant mutation (P104L) in CAV3 (2).

In brief, the cardiac tissue was incubated with cold MeOH, MTBE was added and water was utilized to induce phase separation. The individual fractions containing lipids (top phase), metabolites (lower phase) and proteins (pellet) were then subjected to individual mass spectrometry based workflows. The application of the SIMPLEX workflow pinpointed multiple molecular mechanisms involved in the pathogenesis of functional CAV3-deficiency. In order to maintain the protein homeostasis, the mutant protein is excessively degraded while the integrity of the cellular membranes and cytoskeleton are affected. The increased oxidative stress burden upon P104L mutant CAV3 expression was directly reflected by the altered cardiolipin and plasmalogen levels. Similar trends were observed for TAG species along with a downregulation of glycolytic and TCA cycle intermediates and an accumulation of acylcarnitines. Protein analysis corroborated these findings and indicates a change in substrate utilization as an attempt of the heart to maintain a normal cardiac function. The interconnection provided by the lipidomics, metabolomics and proteomics data revealed the cardiomyopathic phenotype as a severe metabolic disease, not only as a structural one.

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A multi-omics approach to screen for modified hippocampal lipid signaling pathways triggered by lifestyle conditions

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Introduction: Enriched environment (EE) animal studies revealed fundamental changes in the neuronal structure, development, and electro-physiology of hippocampal brain regions whereby EE housed animals also show a better performance in physical and cognitive tests. However, until today, it is hardly possible to correlate these features to differences in the lipidome and proteome composition of the hippocampal region of EE animals.

Materials and Methods: To explore the role of general protein and lipid composition we established an integrative multi-omics approach combining metabolomics, lipidomics and proteomics techniques. We employed the SIMPLEX sample preparation, consists of an MTBE extraction of lipids, proteins and metabolites, as a highly sensitive explorative approach to identify the involved specific lipid signaling pathways. Here we describe the molecular inventory of synaptosome (Syn) (containing axons, wholes synapses) and synaptic junction (SyJ) (the actual membranes where two cells connects and signal transductions is taking place), of pooled C57BL/6J mouse hippocampi (n=5) kept under enriched environment versus standard environment.

Results: Global proteomics analysis identified 2164 proteins from which 57 and 121 proteins revealed a significant up and down regulation, respectively. Significant changes in the proteome were indicated by minimum log2-fold change with a p-value signalling pathway (RESP) was identified as the solely altered lipid messenger pathway with 7 and 10 up and down regulated proteins, respectively, from 6 different protein complexes. Specifically, 2-arachidonoyl glycerol (2-AG) hydrolase (ABDH6) and fatty acid amide hydrolase (FAAH) displayed significant changes. These proteins are responsible for degradation of 2-AG and N-arachidonoyl ethanolamine (AEA) which are the main lipid transmitters partly regulating pain, addiction, cognition, emotionality and neurodegeneration in the brain. Expression change of these enzymes were validated by immunocytochemistry and western blot analysis. Targeted nano-LC high-resolution lipidomics experiments underscored these findings by displaying a significant reduction of the main RESP lipid neurotransmitter 2-AG (from 139.41 $\pm$ 27.18 vs. 101.73 $\pm$ 14.41 nM/mg protein, p= 0.046, non-EE vs. EE, respectively) whereby AEA remained unchanged in the synaptosome. Additionally, functional electrophysiological measurements supported our findings by displaying a reduced action potential of the inhibitory postsynaptic current of the mice grown in EE.

Conclusions: In conclusion, the inhibitory retrograde endocannabinoid pathway, which is crucial for memory and learning, is down-regulated on protein and lipid messenger level in enriched environment mice, leading potentially to a higher neuronal plasticity of EE housed animals. Using SIMPLEX, we were able to demonstrate that an integrative multi-omics approach is a highly valuable tool suitable to provide detailed insights of neuronal metabolic mechanism of interest under even soft or non-invasive lifestyle interventions.

The temporal generation of oxidized phospholipids and fatty acids during physiological blood clotting reveals complex interconnected networks, controlled by cells and enzymes

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Blood coagulation is essential for physiological haemostasis. The temporal generation of bioactive lipids including eicosanoids and procoagulant phospholipids termed enzymatically-oxidized phospholipids (eoxPLs), during physiological clot formation is uncharacterised. Here, we reveal using a lipidomic approach, formation of large numbers of eoxPL and oxidized fatty acid molecular species during ex vivo clotting (0 – 4 hours) of human blood. To mimic physiological clotting, freshly drawn blood was collected with sodium citrate and corn trypsin inhibitor, then re- calcified and allowed to clot via the extrinsic pathway (tissue factor (TF)-dependent activation) at 37 °C for 70 different eoxPL species were detected to form, including phosphatidylethanolamines (PE) and phosphatidylcholine (PC) containing diverse oxidized moieties derived from PUFA. Similarly, in serum, >40 diverse oxygenated fatty acids (oxFA) were formed. A temporal generation of specific families of eoxPLs and oxFA was seen based on the order of cell and enzyme activation. For example, 15 or 5-HETE-PEs formed by eosinophils or neutrophils increased within 1 min activation. Platelet COX-1-derived lipids, including DiEHEDA-PEs, DiEHEDA, and TXB2, were generated following 2 - 5 minutes, while, platelet 12-LOX-derived products, including free and esterified 12-HETE, appeared considerably later, at 60 minutes. In contrast, shorter chain/truncated oxPLs were present at baseline but rapidly disappeared, following a 2-minute stimulation. Their disappearance negatively correlates with 12- or 15-HETE formation, suggesting that truncated oxPLs form via different pathways. In contrast, free 11-, 12- and 15-HETE as well as DiEHEDA positively correlate with their eoxPL forms, indicating that their synthesis is regulated in a similar manner. These findings demonstrate the temporal formation of procoagulant eoxPLs by blood cells that initiate the extrinsic pathway and reveal a complex coordination of cell activation during this process. The relative importance of different cellular eoxPL species to the effective function of a human clot remains to be determined. *This research was funded by the Wellcome Trust and European Research Council.*

Nano-LC/NSI MS refines lipidomics by enhancing lipid coverage, measurement sensitivity, and linear dynamic range

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P34 PRIZE

Nano-liquid chromatography (nLC) - nano-electrospray (NSI) is one of the cornerstones of mass spectrometry based bioanalytics. Nevertheless, the application of nLC is not prevailing lipid analyses so far. In this study, we established a reproducible nLC separation for global lipidomics and describe the merits of using such a miniaturized system for lipid analyses. In order to enable comprehensive lipid analyses that is not restricted to specific lipid classes, we particularly optimized sample preparation conditions and reversed phase separation parameters. We further benchmarked the developed nLC system to a commonly used high flow HPLC/ESI MS system in terms of lipidome coverage and sensitivity. The comparison revealed an intensity gain between two and three orders of magnitude for individual lipid classes and an increase in the linear dynamic range of up to two orders of magnitude. Furthermore, the analysis of the yeast lipidome using nLC/NSI resulted in more than a 3-fold gain in lipid identifications. All in all, we identified 447 lipids from the core phospholipid lipid classes (PA, PE, PC, PS, PG and PI) in *Saccharomyces cerevisiae.*

Regulation of lipid metabolism by GATA6: an integrated 'omics approach

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P35 **PRIZE**

The transcription factor GATA6 was recently recognised as a master regulator of the phenotype and function of peritoneal resident macrophages (pResMΦ), whose deficiency results in dysregulated proliferative renewal during homeostasis and altered inflammatory responses, associated with delays in resolution.

Herein, I show using microarray analysis that mice with a myeloid deficiency of *Gata6* (*Gata6*-KO^{mye} mice) have significant changes in genes associated with lipid metabolism, in particular sphingolipids (SL). Three of the most notable alterations were the downregulation of genes responsible for the degradation of glucosylceramides (GlcCer) and sphingomyelins (SM) (*Gba2* and *Smpd1*), as well as a gene involved in the regulation of lysosomal pH, and consequently, lysosomal function. To characterise the metabolic defect, I compared the lipidome of *Gata6*-KO^{mye} and wild-type (WT) pResMΦ using a high-resolution LC-MS based global lipidomics approach and replicated my findings using targeted LC-MS/MS. SL showed a high percentage of changes (25% of all lipids levels were altered significantly) with a marked increase in *Gata6*-KO^{mye} pResMΦ. SL are important constituents of the plasma membrane in eukaryotes and, as second messengers, modulate apoptosis, cell proliferation and differentiation. Targeted lipidomics by LC-MS/MS showed that the most significantly-increased molecular species were long chain GlcCer and SM. This linked with downregulation of *Gba2*, *Ctse* and *Smpd1*, observed during the microarray analysis, and was confirmed by suppressing these pathways, in RAW 264.7 cells, using knockdown (shRNA) approaches. Accumulation of SL metabolites in tissues is implicated in a plethora of pathophysiological complications such as development of neurological dysfunctions, atherosclerosis, diabetes, and heart failure, whilst in cells it correlates with defects in cell migration, cholesterol traffic and efflux, lipid transport, cell activation, proliferation and apoptosis.

Overall, I concluded that GATA6 regulates lipid metabolism in pResMΦ, on both a transcriptional and metabolic level, with particular focus on SL levels. Furthermore, there is indication that this regulation may at least in part control phenotype and function in pResMΦ through altering SL signalling. Thus, my studies propose GATA6 as a new lipid-regulating transcription factor and highlight the importance of lipid regulation in pResMΦ biology.

Biomarker research and short time chromatography: a utopia

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P36 PRIZE

Introduction:
In biomarker research short analysis times are preferred, which are limited by problems like matrix effects and coelution of isobaric compounds. For this reason, run-times often exceed 30 or even 50 minutes. Our aim is to implement an untargeted method for high-throughput screening, reducing drifts in instrumental response, retention time shifts and improving sample stability.

Methods:
We developed a chromatographic method with a sub 2 micrometre C8 column and a binary gradient to cover the lipidome ranging from polar lipids to highly apolar triacylglycerides and cholesteryl esters in biological samples after liquid-liquid extraction. The chromatographic system was coupled with quadrupole-time-of-flight mass spectrometry for lipid identification.

Results:
The obtained chromatographic resolution allowed the separation of isobaric lipids and reduced ion suppression effects. Combined with high resolution mass spectrometry we could identify lipids from over 15 different lipid classes. The resulting acquisition time was 17 minutes per sample and polarity, simplifying the measurement of large sample numbers and improving system stability. Hence we achieved a coefficient of variation (CV) below 15% for the peak area of 220 lipids in human plasma at repeated extractions, with 205 of these having a CV below 10%. Additionally, the average CV of the retention times was 0.1%. We successfully applied the method on plasma, serum and cell culture samples as well as several different mouse tissue types. For the latter, we could show the importance of the sampling location of mouse kidney, heart and liver for the reproducibility of the lipid profile.

Conclusions:
The developed method can be applied for high-throughput lipidomics and biomarker discovery research in biological samples.

Novel Aspect:
LC-HRMS analysis of biological samples in 17 minutes with low matrix effects, adequate peak resolution, lipid identification and method reproducibility.

Lipid Compass

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P37

Lipidomics as a discipline has seen a steady increase in research output throughout the last decade. With the advent of high-throughput lipidomics platforms based on high resolution mass spectrometry, the need for a central, well connected and comprehensive resource for both experimental and computational scientists has increased.

With Lipid Compass, we want to simplify the exploration of the lipidomic structural space from different angles, following the structural hierarchy, as established by LipidMaps and the proposed extension to further levels based on high-resolution MS technologies, as proposed by Liebisch *et al.* [1], which has been implemented in the LipidHome database for theoretical lipids [2] and in Swiss Lipids [3]. Based on the hierarchy of in-silico lipid structures for two main lipid categories in LipidHome, currently Glycerolipids and Glycerophospholipids, we plan to extend the available categories with further lipid categories in the near future.

Every theoretical lipid in the database will have an associated level of confidence according to its identification status, e.g. being mentioned in the literature, having evidence from actual experimental data on the MS/MS level, or even having quantitative data available. Additional information on e.g. species and tissue where lipids have been identified are linked in the database for cross-cutting analysis and queries, together with external links to PubChem, Chebi and other relevant resources.

To simplify the submission of experimental data and thus evidence for theoretical lipids in the database, we will integrate with the data deposition workflow established by MetaboLights [4] using mzTab-M with customizations for lipidomics as an additional format for summary and detail information on lipids.

Lipid Compass will furthermore be an integration point for multiple lipid-related web services, such as LUX Score and LipidXplorer as part of the Lipidomics informatics for life-science (LIFS) project [5].

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Identification of key lipids critical for platelet activation by comprehensive analysis of the platelet lipidome

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P38 PRIZE

Platelet integrity and function critically depend on lipid composition. However, the lipid inventory in platelets was hitherto not quantified. Here, we examined the lipidome of murine platelets using lipid-category tailored protocols on a quantitative lipidomics platform. We could show that the platelet lipidome comprises almost 400 lipid species and covers a concentration range of 7 orders of magnitude. A systematic comparison of the lipidomics network in resting and activated murine platelets, validated in human platelets, revealed that <20% of the platelet lipidome is changed upon activation, involving mainly lipids containing arachidonic acid. Sphingomyelin phosphodiesterase-1 (Smpd1) deficiency resulted in a very specific modulation of the platelet lipidome with an order of magnitude upregulation of lysosphingomyelin (SPC), and subsequent modification of platelet activation and thrombus formation. In conclusion, this first comprehensive quantitative lipidomic analysis of platelets sheds light on novel mechanisms important for platelet function, and has therefore the potential to open novel diagnostic and therapeutic opportunities.

Notes

Notes

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