joint
1st ILS CONFERENCE and 7th LIPIDOMICS FORUM

OCTOBER 5th – 8th · 2021
IN THE WORLD HERITAGE OF
REGENSBURG · GERMANY

CLINICAL LIPIDOMICS
SIGNALING LIPIDOMICS
STANDARDIZATION

INTERNATIONAL LIPIDOMICS SOCIETY

LIPIDOMICS FORUM

AbstractBook

https://ils2021.org

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Welcome to Regensburg

On behalf of the organizing committee, we would like to welcome you to the Joint 1st International Lipidomics Society (ILS) annual conference and 7th Lipidomics Forum, at the University of Regensburg. We hope you had a pleasant and safe trip to Regensburg.

It is our great pleasure that the progress in SARS-CoV-2 vaccination efforts allows us to host an in-person meeting. For participants who cannot attend on site, we will provide a live stream of the talks and discussion. The meeting brings together an international group of participants from all career levels and from various scientific disciplines to present cutting-edge research in a collegial atmosphere. Due to your excellent contributions, we were able to set up 10 exciting sessions with more than 40 talks and more than 40 posters. We look forward to stimulating presentations and discussions that will already start with various workshops and a fascinating opening lecture. The ILS is also delighted to offer and support three dedicated short talk slots for early-career female researchers in lipidomics, in accordance with the ILS statement on diversity and inclusion.

We are especially indebted to the German Research Foundation (DFG) and our industry partners for their generous support, which makes this meeting possible.

We hope that you will enjoy the meeting and the World Heritage of Regensburg.

Ralph Burkhardt, Gerhard Liebisch and Silke Matysik,
Local Organizing Committee

Organizing Committee

Local Organizing Committee

Ralph Burkhardt

Gerhard Liebisch

Silke Matysik

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

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Abstract Selection Early Career Females in Lipidomics Category

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Lipidomics Forum Organizers

Robert Ahrends, University of Vienna, Austria

Nils Hoffmann, Bielefeld University, Germany

Dominik Kopczynski, University of Vienna, Germany

Dominik Schwudke, Research Center Borstel, Germany

LIFS Training

Nils Hoffmann, Bielefeld University, Germany

Jacobo Miranda Ackerman, MPI-CBG Dresden, Germany

Fadi Al Machot, Research Center Borstel, Germany

Daniel Krause, Research Center Borstel, Germany

Dominik Kopczynski, University of Vienna, Germany

Scientific Advisory Board

Harald Köfeler, Medical University Graz, Austia

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Willi Griffiths, Swansea, UK

Kim Ekroos, Helsinki, Finland

Orientation in Regensburg

MEETING VENUE

University Regensburg, Grand Lecture Hall (Audimax)

Universitätsstraße 31 · 93053 Regensburg

PUBLIC TRANSPORTATION

There are several bus stops nearby the University (see Campus Map next page).

Timetable and maps are available at https://www.rvv.de/tourists.

WIFI

Please use open access:

CONFERENCE DINNER

The conference dinner will take place at **Leerer Beutel**

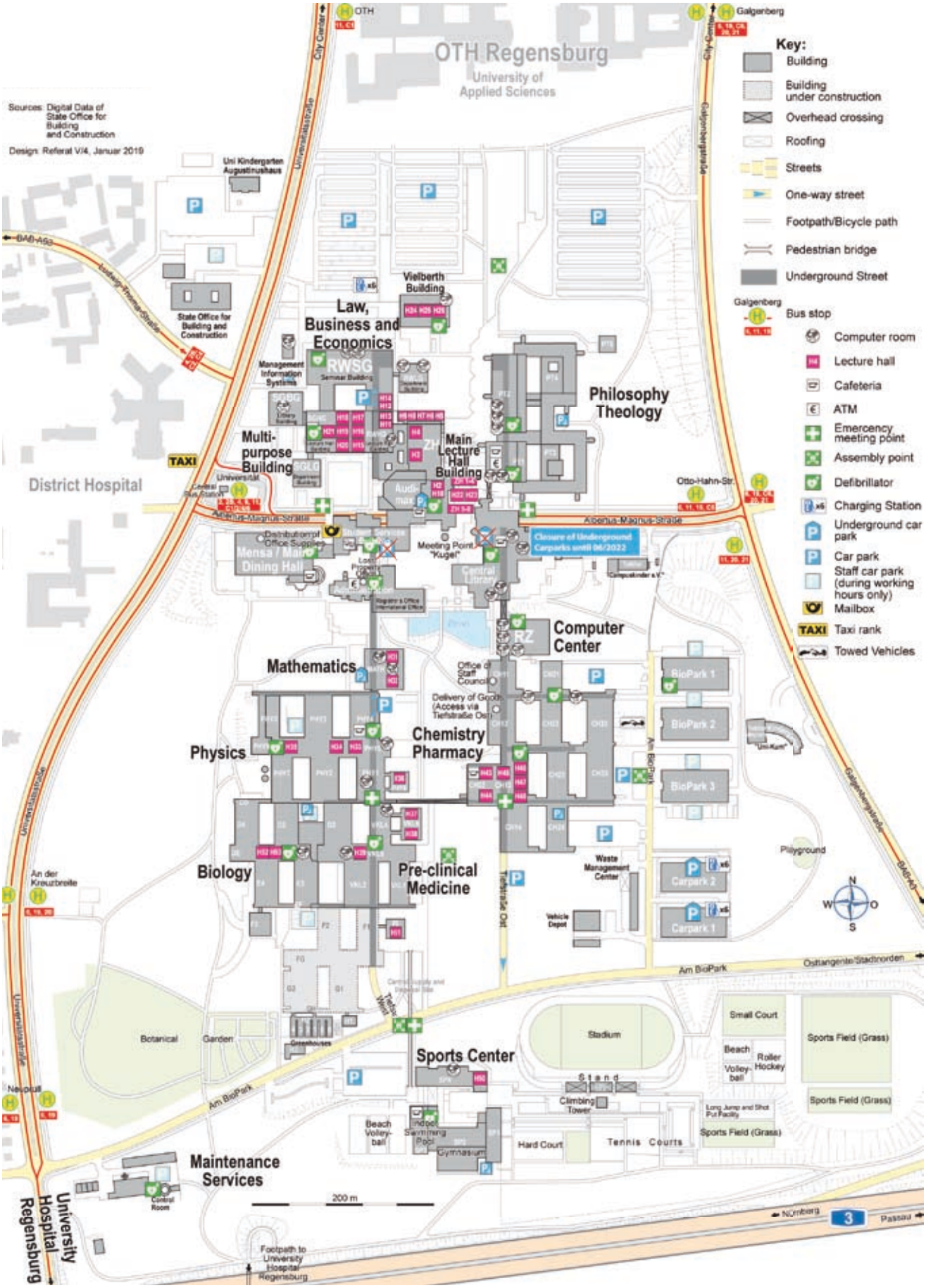
https://www.leerer-beutel.de

Bertoldstraße 9 · 93047 Regensburg

on 7th October, starting at 19:30.

Please bring your badge with you.

UNIVERSITY REGENBURG



Program at Glance

Tuesday | **October 5**

9:00 – 12:00	**LIFS I: LIFS Training Session I** Session Chair: Nils Hoffmann	Lecture Hall 2 (H2)
	LipiTUM Workshop	Grand Lecture Hall (Audimax)
12:00 – 13:00	LUNCH	
13:00 – 15:00	**ILS Interest Group Presentations**	Grand Lecture Hall (Audimax)
	LIFS II: LIFS Training Session II Session Chair: Nils Hoffmann	Lecture Hall 2 (H2)
15:00 – 15:30	BREAK	
15:30 – 17:00	**Lipidomics Standardization Initiative** Update and Discussion	Grand Lecture Hall (Audimax)
17:00 – 18:00	BREAK	
18:00 – 19:00	**OPENING LECTURE** **Membrane lipidomics at the nexus of biophysics and cell physiology** Ilya Levental, *University of Virginia, Charlottesville, Virginia, USA* Session Chair: Dominik Schwudke	Grand Lecture Hall (Audimax)
19:00 – 21:00	WELCOME MIXER	

Wednesday | **October 6**

Grand Lecture Hall (Audimax)

SESSION 1 — **Lipid Metabolism**
Session Chairs: Emanuela Camera, Ralph Burkhardt

9:00 – 9:30 — KEYNOTE
Beyond „good cholesterol": Lipidomic analysis of high-density lipoprotein (HDL)
Anatol Kontush, *INSERM, Sorbonne University and Pitié-Salpétrière Hospital, Paris, France*

9:30 – 9:50 — EARLY CAREER FEMALES IN LIPIDOMICS CATEGORY
Ontogeny of plasma lipid metabolism in pregnancy and early childhood: a longitudinal population study
Satvika Burugupalli, *Metabolomics Laboratory, Baker Heart and Diabetes Institute, Australia*

9:50 – 10:10 — **Intra-tumoral heterogeneity of lipid metabolism in glioblastoma**
Sweta Parik, *VIB-KU Leuven Center for Cancer Biology, VIB, Leuven, Belgium; VIB Center for Inflammation Research, Brussels, Belgium; Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Leuven, Belgium; Vrije Universiteit Brussel, Brussels, Belgium*

10:10 – 10:30 — **What is the lowest common denominator of the mammalian lung lipidome? A cross-species study.**
Daniel Krause, *Division of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany*

10:30 – 11:00 — BREAK

SESSION 2 — **Clinical Lipidomics – Technologies**
Session Chairs: Cristina Coman, Christer Ejsing

11:00 – 11:30 — KEYNOTE
Lipid class separation 2 mass spectrometry workflows in high-throughput lipidomic quantitation
Michal Holčapek, *University of Pardubice, Pardubice, Czech Republic*

11:30 – 11:50 — **Using reference materials to generate biological reference ranges in lipidomics**
Federico Torta, *NUS, Singapore*

11:50 – 12:10 — EARLY CAREER FEMALES IN LIPIDOMICS CATEGORY
A novel lipid droplet specific LC-MSn workflow for investigating therapy-resistance mechanisms in human colon cancer
Katharina Hohenwallner, *Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria*

12:10 – 12:30 — **Accumulation of dihydrosphingolipids and neutral lipids are related to steatosis and fibrosis damage both in human and animal models of NAFLD**
OSCAR PASTOR, *UCA-CCM, Servicio de BioquímicaClínica. Hospital Universitario Ramón y Cajal-IRYCIS. Madrid; Grupo de Lípidos. Servicio de Bioquímicalnvestigación. Ramón y Cajal-IRYCIS. Madrid, Spain*

12:30 – 14:00 — LUNCH

13:30 – 14:00 — **LUNCH WORKSHOP**

ThermoFisher SCIENTIFIC

SESSION 3

Shotgun Lipidomics
Session Chairs: Marcus Höring, *Ilya Levental*

14:00 – 14:30 **KEYNOTE**
Update on multi-dimensional mass spectrometry-based shotgun lipidomics and its biological/biomedical applications
Xianlin Han, *Barshop Institute, UT Health, San Antonio, TX, USA*

14:30 – 14:50 **Flexibility of a mammalian lipidome — Insights from mouse lipidomics**
Christian Klose, *Lipotype GmbH, Germany*

14:50 – 15:10 **Lipidomic signatures of NAFLD progression**
Olga Vvedenskaya, *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; Spectroswiss, Lausanne, Switzerland*

15:10 – 15:30 **Computational stratification and subtyping on NAFLD liver lipidomics**
Tim Daniel Rose, *LipiTUM, Chair of Experimental Bioinformatics, Technical University of Munich, Germany*

15:30 – 16:00 BREAK

SESSION 4

Clinical Lipidomics – Current Applications
Session Chairs: Irina Alecu, Kim Ekroos

16:00 – 16:30 **KEYNOTE**
Plasma ceramides as biomarkers of cerebrovascular disease and dementia
Michelle Mielke, *Mayo Clinic, Rochester, Minnesota, USA*

16:30 – 16:50 **Comprehensive lipidomic profiling along the clinical patient journey by laser assisted rapid evaporative ionisation mass spectrometry – from cancer screening to MS-guided surgery**
Zoltan Takats, *Imperial College London, United Kingdom*

16:50 – 17:10 **Altered Plasma, Urine, and Tissue Profiles of Sulfatides and Sphingomyelins in Renal Cell Carcinoma Patients**
Robert Jirásko, *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic*

17:10 – 17:30 **Fingerprints and footprints of sebum associated with profiles of abundance of epidermal lipids in the stratum corneum: relevance in skin physiology and in atopic dermatitis.**
Emanuela Camera, *San Gallicano Dermatological Institute - IRCCS, Rome, Italy*

17:30 **PRESENTATION OF REMOTE POSTERS**

18:00 – 21:00 POSTER SESSION & BEER

Thursday | **October 7**

Grand Lecture Hall (Audimax)

SESSION 5

Structural Lipidomics
Session Chairs: Kai Schuhmann, Michal Holčapek

9:00 – 9:30 **KEYNOTE**
Integrating Hydrophilic Interaction Chromatography, Trapped Ion Mobility, and Isomer Resolving MS/MS Enables Fast and In-Depth Lipidomic Profiling
Yu Xia, *Department of Chemistry, Tsinghua University, Beijing, China*

9:30 – 9:50 **Mapping of alkyl and alkenyl ether lipids in RP-LC-MS experiments**
Jakob Koch, *Institute of Human Genetics, Medical University of Innsbruck, Austria*

9:50 – 10:10 **HILIC meets MS imaging: CCS values obtained by chromatographic separation coupled to trapped ion mobility-MS for unequivocal assignment of phospholipids in MALDI-MS-imaging**
Ansgar Korf, *Bruker Daltonics GmbH & Co. KG, Bremen, Germany*

10:10 – 10:30 **Identification and quantitation of glycolipidicrhamnolipids by supercritical fluid chromatography-mass spectrometry and charged aerosol detection**
Anna Lipphardt, *Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany*

10:30 – 11:00 BREAK

SESSION 6

Sphingolipids Signaling/Health and Disease
Session Chairs: Stefanie Rubenzucker, Gerhard Liebisch

11:00 – 11:30 **KEYNOTE**
Ceramides and the two phases of lipotoxicity?
Scott Summers, *University of Utah, Salt Lake City, Utah, USA*

11:30 – 11:50 **A REFERENCE MAP OF SPHINGOLIPIDS IN MURINE TISSUES**
Sneha Muralidharan, *Singapore Lipidomics Incubator, Life Sciences Institute, National University of Singapore, Singapore*

11:50 – 12:10 **Probing the platelet lipidome and identifying key lipids critical for platelet activation by comprehensive lipidomics**
Robert Ahrends, *Department of Analytical Chemistry, University of Vienna, Vienna, Austria*

12:10 – 12:30 **Gangliosides Characterization and Isomer Separation using SLIM-based High Resolution Ion Mobility (HRIM)-Mass Spectrometry (MS)**
Komal Kedia, *MSD, West Point, PA 19486 USA*

12:30 – 14:00 LUNCH

13:30 – 14:00 **LUNCH WORKSHOP**
Lipid Characterization with EAD using the ZenoTOF 7600 system
Christian Baumann, *Accurate Mass Workflows, SCIEX*

SESSION 7

Bile Acids and Lipid Signaling
Session Chairs: Bing Peng, Justine Bertrand-Michel

14:00 – 14:30 **KEYNOTE**
Bile acid signaling and the regulation of metabolic control
Christian Wolfrum, *Institute of Food Nutrition and Health, ETH Zürich, Zürich, Switzerland*

14:30 – 14:50 **Short chain fatty acids and bile acids in human faeces are associated with the intestinal cholesterol conversion status**
Silke Matysik, *Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany*

14:50 – 15:10 **CRISPR/Cas-9-mediated inactivation of soluble epoxide hydrolase N-TIe domain, a lipid phosphate phosphatase, promotes thermogenesis through peroxisome proliferator activated receptor gamma.**
Thomas Duflot, *Normandy University, UniRouen, Inserm UMR1096 EnVI, Rouen, France;Department of Pharmacology, Rouen University Hospital, Rouen, France; Laboratory of Pharmacokinetics, Toxicology and Pharmacogenetics, Rouen University Hospital, Rouen, France*

15:10 – 15:30 **Assessing membrane fluidity of antibiotic-resistant Staphylococcus aureus using an RPLC-IM-MS method for isomeric phospholipid separations**
Kelly M. Hines, *Department of Chemistry, University of Georgia, Athens, GA, United States of America*

15:30 – 16:00 BREAK

SESSION 8

Lipid Biomarker
Session Chairs: Olga Vvedenskaya, Zoltan Takats

16:00 – 16:30 **KEYNOTE**
Lipidomic-Based Insights into Disease Mechanisms:
A Targeted Lipidomics Discovery Pipeline for Network Analysis
Irina Alecu, *University of Ottawa, Ottawa, Ontario, Canada*

16:30 – 16:50 **Multiomics of synaptic junctions reveals altered lipid metabolism and signaling upon environmental enrichment**
Cristina Coman, *Department of Analytical Chemistry, University of Vienna, 1090 Wien, Austria*

16:50 – 17:10 **Modified lipids and lipid-protein adducts as pro-inflammatory markers in obesity and related complications**
Patricia Prabutzki, *Leipzig University, Faculty of Medicine, Institute for Medical Physics and Biophysics, Leipzig, Germany*

17:10 – 17:30 **EARLY CAREER FEMALES IN LIPIDOMICS CATEGORY**
Chemical cartography of localized lipidomic alterations during infectious diseases
Laura-Isobel McCall, *Department of Chemistry and Biochemistry, University of Oklahoma, USA*

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19:30 – 23:00 CONFERENCE DINNER

Friday | **October 8**

Grand Lecture Hall (Audimax)

SESSION 9 — **Standards and Tools for Lipid Data Processing & Analysis**
Session Chairs: Tim Daniel Rose, Robert Ahrends

9:00 – 9:30 — **KEYNOTE**
Lipid molecular timeline profiling reveals diurnal crosstalk between the liver and circulation
Christer Ejsing, University of Southern Denmark, Odense, Denmark & EMBL, Heidelberg, Germany

9:30 – 9:50 — **Lipid Creator workbench to probe the lipidomic landscape**
Dominik Kopczynski, Department of Analytical Chemistry, University of Vienna, Austria

9:50 – 10:10 — **Integration of lipid specific fragmentation rules into SIRIUS**
Kai Dührkop, *Department of Bioinformatics, Faculty of Mathematics and Computer Science, Friedrich Schiller University, Jena, Germany*

10:10 – 10:30 — **Smart Peak Automates Metabolomics, Fluxomic, and Lipidomic Data Processing**
Douglas McCloskey, *Technical University of Denmark, Denmark*

10:30 – 11:00 — BREAK

SESSION 10 — **Data Integration**
Session Chairs: Dominik Kopczynski, Dominik Schwudke

11:00 – 11:30 — **KEYNOTE**
LION/web: a web-suite for lipidomics data analysis
Martijn Molenaar, *Alexandrov Group, EMBL, Heidelberg, Germany*

11:30 – 11:50 — **Automation of Large-Scale Lipidomics Workflow Management, Data Processing, QA/QC and Reporting**
Bo Burla, *National University of Singapore, Singapore*

11:50 – 12:10 — **The LIFS tools and workflows for mass-spectrometry based lipidomics**
Nils Hoffmann, *Center for Biotechnology (CeBiTec), Bielefeld University, Germany*

12:10 – 12:30 — **Investigating Global Lipidome Alterations with the Lipid Network Explorer**
Nikolai Köhler, *LipiTUM, Chair of Experimental Bioinformatics, TUM School of Life Sciences, Technical University of Munich, Freising, Germany*

12:30 – 12:45 — **AWARD CEREMONY**
Poster and Presentation Prizes

12:45 – 13:00 — **Presentation of ILS 2022**
Session Chair: Erin S. Baker

13:00 – 14:00 — LUNCH AND DEPARTURE

Abstracts of Short Talks

SESSION 1
LIPID METABOLISM

Session Chairs: Emanuela Camera, Ralph Burkhardt

9:00 – 9:30 **KEYNOTE**
Beyond „good cholesterol": Lipidomic analysis of high-density lipoprotein (HDL)
Anatol Kontush, *INSERM, Sorbonne University and Pitié-Salpétrière Hospital, Paris, France*

9:30 – 9:50 EARLY CAREER FEMALES IN LIPIDOMICS CATEGORY
Ontogeny of plasma lipid metabolism in pregnancy and early childhood: a longitudinal population study
Satvika Burugupalli, *Metabolomics Laboratory, Baker Heart and Diabetes Institute, Australia*

9:50 – 10:10 **Intra-tumoral heterogeneity of lipid metabolism in glioblastoma**
Sweta Parik, *VIB-KU Leuven Center for Cancer Biology, VIB, Leuven, Belgium; VIB Center for Inflammation Research, Brussels, Belgium; Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Leuven, Belgium; Vrije Universiteit Brussel, Brussels, Belgium*

10:10 – 10:30 **What is the lowest common denominator of the mammalian lung lipidome? A cross-species study.**
Daniel Krause, *Division of Bioanalytical Chemistry, Research Center Borstel, Borstel, Germany*

10:30 – 11:00 BREAK

Ontogeny of plasma lipid metabolism in pregnancy and early childhood: a longitudinal population study

Satvika Burugupalli[1] | satvika.burugupalli@baker.edu.au
Adam T. Alexander Smith[1], Gavriel Olshansky[1], Kevin Kevin.huynh[1], Corey Giles[1], Sudip Paul[1], Anh Nguyen[1], Thy Duong[1], Natalie Mellett[1], Michelle Cinel[1], Sartaj Ahmad Mir[2,3], Li Chen[3,4], Markus R. Wenk[2,3], Neerja Karnani[2,4], Fiona Collier[5,6,7], Richard Saffery[7,8], Peter Vuillermin[5,6,7], Anne-Louise Ponsonby[7,8], David Burgner[7,8], Peter J. Meikle[1]

[1] Metabolomics Laboratory, Baker Heart and Diabetes Institute, Australia
[2] Department of Biochemistry, Yong Loo Lin School of Medicine, National University of Singapore, Singapore
[3] Singapore Lipidomics Incubator, Life Sciences Institute, National University of Singapore, Singapore
[4] Singapore Institute for Clinical Sciences, A*STAR, 30 Medical Drive, Singapore, 117609, Singapore
[5] School of Medicine, Deakin University, Australia
[6] Child Health Research Unit, Barwon Health, Australia
[7] Murdoch Children's Research Institute, Royal Children's Hospital, Australia
[8] The Florey Institute of Neuroscience and Mental Health, Australia

T 01

Background:

There is mounting evidence that in *utero* and early life exposures may predispose an individual to metabolic disorders in later life; and dysregulation of lipid metabolism is critical in such outcomes. However, there is limited knowledge about lipid metabolism and factors causing lipid dysregulation in early life that could result in adverse health outcomes in later life. In this study, we aim to understand the lipid metabolism in pregnancy, and from birth to four years.

Methods:

We performed comprehensive lipid profiling of 1074 mother-child dyads in the Barwon Infant Study (BIS), a population based pre-birth cohort and measured 776 distinct lipid species across 42 lipid classes using ultra high-performance liquid chromatography (UHPLC). We measured lipids in 1032 maternal serum samples at 28 weeks' gestation, 893 cord serum samples at birth, 793, 735, and 511 plasma samples at six, twelve months, and four years, respectively.

Results:

The lipidome differed between mother and newborn and changed markedly with increasing postnatal age. Cord serum was enriched with long chain poly-unsaturated fatty acids (LC-PUFAs), and corresponding cholesteryl esters relative to the maternal serum. Alkenyl-phosphatidylethanolamine species containing LC-PUFAs increased with postnatal age, whereas the corresponding lysophospholipids and triglycerides decreased as the child reached four years. At birth, majority of the cord serum lipids were strongly associated with gestational age and birth weight, with most lipids showing opposing associations. Each mode of birth showed an independent association with cord serum lipids.

Conclusions:

There were marked changes in the plasma lipidome over the first four years of life. This study sheds light on lipid metabolism in infancy and early childhood and provide a framework to define the relationship between lipid metabolism and health outcomes in early childhood.

Intra-tumoral heterogeneity of lipid metabolism in glioblastoma

Sweta Parik[1,2,3,4] | sweta.parik@kuleuven.be
Juan Fernandez-Garcia[1,2], Francesca Lodi[1,5], Frederik De Smet[6], Gabriele Bergers[1,7], Diether Lambrechts[1,5], Jo Van Ginderachter[2,4], Sarah-Maria Fendt[1,3]

1 VIB-KU Leuven Center for Cancer Biology, VIB, Leuven, Belgium
2 VIB Center for Inflammation Research, Brussels, Belgium
3 Department of Oncology, KU Leuven and Leuven Cancer Institute (LKI), Leuven, Belgium
4 Vrije Universiteit Brussel, Brussels, Belgium
5 Department of Human Genetics, KU Leuven, Leuven, Belgium
6 Department of Imaging and Pathology, KU Leuven, Leuven, Belgium
7 UCSF Comprehensive Cancer Center, Department of Neurological Surgery, UCSF, San Francisco, CA, USA.

T 02

Glioblastoma accounts for the majority of adult diffuse gliomas and is the mostlethal form, with a very high mortality rate. The current standard of care issurgical resection followed by radiotherapy and chemotherapy using thealkylating agent, temozolomide (TMZ). Despite these, the median survival is only 14-17 months with relapse occurring in ~80 % of the cases[1]. Over the pastdecades, the target of glioblastoma research has been to establish therapies thatmight extend the survival of patients but the prospects have so far been bleak.

One of the main hindrances towards the development of effective therapies have been the highly specialized metabolic niche[2] and the very heterogeneous nature of the tumor resident cells[3]. We were therefore, interested in investigating the metabolic heterogeneity in glioblastoma tissues, particularly in terms of their lipid metabolism. To this end, we performed single cell sequencing in tissues from untreated glioblastoma and examined the heterogeneity of lipid metabolism genes, namely fatty acid synthetase (FASN), stearoyl coA desaturase 1 (SCD1), fatty acid desaturase 2 (FADS2) and fatty-acid binding protein 7 (FABP7). Firstly, we observed that lipid metabolic genes display highly heterogeneous expression within cancer cells and a signature indicative of reliance on *de novo* fatty acid synthesis rather than uptake. Indeed, mass spectrometric analysis confirmed that the glioblastoma microenvironment is highly depleted in circulating lipids.

In addition, we observed that the cancer cells classify into distinct clusters, featuring unique fatty acid metabolism gene signatures, again highlighting the primary feature of tumor heterogeneity within glioblastoma. Most interestingly, the fatty acid desaturases SCD1 and FADS2 displayed mutually exclusive expression patterns in particular cancer cell subpopulations. We recently reported that liver and lung cancer cells exploit dependent upregulation of FADS2 and SCD1 in order to confer metabolic flexibility[4]. In concordance with this, we further confirmed that glioblastoma cells have highly elevated FADS2 monodestauration activity, raising the interesting question of the role of FADS2 metabolism in the different cancer cell clusters. GO pathway analysis of the clusters indicate that FADS2 expressing cancer cells have highly upregulated redox metabolism genes, pointing towards a crucial role of FADS2 in regulating cellular redox homeostasis. Altogether, our results identify the intra-tumoral heterogeneity of lipid metabolic genes in glioblastoma, identify specific clusters within the cancer cells and point towards a role of FADS2 in regulating glioblastoma redox metabolism.

1 Genetic and molecular epidemiology of adult diffuse glioma, *Nature Reviews Neurology*
2 Glioblastoma: microenvironment and niche concept. *Cancers*
3 Molecular and cellular heterogeneity: the hallmark of glioblastoma. *Neurosurgical focus*
4 Evidence for an alternative fatty acid desaturation pathway increasing cancer plasticity. *Nature*

What is the lowest common denominator of the mammalian lung lipidome? A cross-species study.

Daniel Krause[1] | dkrause@fz-borstel.de
Dirk Dannenberger[2], Torsten Goldmann[3,4,5], Lars Lunding[6], Fadi Al Machot[7], Julia Schipke[8], Dominik Schwudke[1,4,5]

[1] Division of Bioanalytical Chemistry, Research Center Borstel, Borstel
[2] Research Institute for Farm Animal Biology, Institute of Muscle Biology and Growth, Dummerstorf
[3] Department of Pathology, Research Center Borstel, Borstel
[4] German Center for Infection Research, TTU TB, Borstel
[5] German Center for Lung Research, Airway Research Center North, Borstel
[6] Asthma Exacerbation & Regulation, Research Center Borstel, Borstel
[7] Department of Data Science, Norwegian University of Life Sciences, Ås, Norway
[8] Institute for Functional and Applied Anatomy, Hannover Medical School, Hannover

T 03

Across mammals, the lung is largely conserved in its anatomy and physiology. The process of gas exchange is associated with a specific metabolism and homeostasis of lipids to safeguard proper intake of oxygen and removal of carbon dioxide. Specifically, the maintenance of the pulmonary alveoli is of utmost importance for the lung health. However, the biological constraints on how much the lipidome of the lung can vary to maintain a healthy status remains largely unexplored. Using an integrative approach, we investigate how environmental factors and medical conditions, as well as physiological parameters such as age, gender, diet, and BMI, influence the overall lipidome composition of the lung.

In a cross-species study, we investigated differences in the lipidome between resected and cancer-free control lung tissues of 30 male human adenocarcinoma patients, 16 female mice (C57BL/6) of similar age and weight with genetic mutations affecting the pulmonary surfactant composition, 36 female mice (BALB/c) of similar age but different weights and diets, 68 pigs male/female (German Large White, German Landrace) lipidomes of similar age and weight but different breeds, and 16 male sheep (German Blackheaded Mutton) of similar age and weight.

We catalogued the lung lipidomes of the animal models and human biopsies using the shotgun lipidomics approach in positive and negative ESI mode. More than 500 lipid species from 16 lipid classes were quantified, with a core lipidome of about 150 lipids present in at least 80 % of all specimens, representing the categories of glycerolipids, glycerophospholipids, sphingolipids and sterol lipids.

We outline the similarities and differences between the lung lipidomes using statistical approaches as well as multivariate analysis methods such as PCA, UMAP, Clustering and applied the LUX score to determine lipidome homologies. We were able to clearly separate different biological species and found that the differences between species outweighed the inherent variation within a biological species. We further investigated the effects of different physiological parameters such as age, diet, and genetic composition on the lipidome to provide a better data base for translational research in lung diseases.

SESSION 2
CLINICAL LIPIDOMICS – TECHNOLOGIES

Session Chairs: Cristina Coman, Christer Ejsing

11:00 – 11:30 **KEYNOTE**
Lipid class separation 2 mass spectrometry workflows in high-throughput lipidomic quantitation
Michal Holčapek, *University of Pardubice, Pardubice, Czech Republic*

11:30 – 11:50 **Using reference materials to generate biological reference ranges in lipidomics**
Federico Torta, *NUS, Singapore*

11:50 – 12:10 EARLY CAREER FEMALES IN LIPIDOMICS CATEGORY
A novel lipid droplet specific LC-MSn workflow for investigating therapy-resistance mechanisms in human colon cancer
Katharina Hohenwallner, *Institute of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Vienna, Austria*

12:10 – 12:30 **Accumulation of dihydrosphingolipids and neutral lipids are related to steatosis and fibrosis damage both in human and animal models of NAFLD**
OSCAR PASTOR, *UCA-CCM, Servicio de BioquímicaClínica. Hospital Universitario Ramón y Cajal-IRYCIS. Madrid; Grupo de Lípidos. Servicio de BioquímicaInvestigación. Ramón y Cajal-IRYCIS. Madrid, Spain*

12:30 – 14:00 LUNCH

13:30 – 14:00 **LUNCH WORKSHOP**

Using reference materials to generate biological reference ranges in lipidomics

Federico Torta[1] | bchfdtt@nus.edu.sg
Nils Hoffmann[2], John Bowden[3], Kim Ekroos[4], Robert Ahrends[5], Markus R. Wenk[1]

[1] NUS, Singapore
[2] Universität Bielefeld
[3] University of Florida
[4] Lipidomics Consulting Ltd
[5] University of Vienna

T 04

For a lipid to be useful in the clinic, physicians must know its levels in a healthy person. Lipidomics researchers are working toward filling this gap of knowledge through a collegial effort. Recently, members of several research groups specialized in various lipid chemistries met to find how to better harmonize studies of the human plasma lipidome. The group settled on ceramides as the first class of molecules to be measured, as they have roles in disease and are thought to be abundant, stable and easy to isolate. A ring trial was launched, distributing the NIST SRM 1950 plasma, and 3 other new reference materials, to about 30 participant labs around the world. The goal was to measure the absolute concentration of four selected ceramides. This exercise allowed us to learn more about inter-assay and inter-lab differences, how data analysis might impact final results and it extended the findings obtained from previous similar initiatives.

More trials are following, with the aim of measuring absolute concentrations of other lipid classes in reference materials. After this first stage, phase two of the project will involve collecting samples from different human cohorts and measure the same lipids, generating reference ranges in healthy and disease conditions, driving a translation towards the clinical use of lipidomics.

A novel lipid droplet specific LC-MSn workflow for investigating therapy-resistance mechanisms in human colon cancer

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T 05

Lipid droplets (LDs) are cellular storage organelles with a hydrophobic core of neutral lipids, above all triacylglycerol (TG) and cholesterol esters (CE) and surrounded by a phospholipid monolayer. They are involved in many cellular regulations and metabolic activities and therefore characterized by highly dynamic changes in number, size, distribution, and response to different stimuli. In cancer cells LDs are often extensively present and according to previous investigations involved in therapy resistance mechanisms. In this work, tailored intervention of the cellular lipid metabolism using triacsin C and oleic acid of an acquired BOLD-100-resistant (KP1339, sodium trans-[tetrachloridobis(1H-indazole)ruthenate(iii)]) colon cancer cell line (HCT116) followed by lipid profiling was performed to gain better insights into metabolic routes and turnover of LDs. In a preceding study significantly higher amounts of LDs could be observed in the resistant cell model compared to parental cells using flow cytometry with Bodipy 493/503 as specific marker, these findings served as starting point for the presented follow-up experiment. LDs are isolated using a commercially available kit (Cell Biolabs) and further extracted with an MTBE-based two-phase method. Targeted and non-targeted lipidomics are performed by reversed-phase high-resolution mass spectrometry (RP-HRMS; Orbitrap ID-X, Thermo-Fisher). In house-produced ^{13}C-labeled internal yeast (*Pichia pastoris*) standards are added for accurate qualitative and quantitative lipid annotation. The lipidomic profiling approach allows to confirm a pronounced influence of the BOLD-100 treatment on the neutral TGs and phosphatidyl choline remodelling of LDs. We are currently optimizing the analytical workflow to resolve the full fatty acid pattern of TGs using the MS^n capability of the MS-instrument.

Accumulation of dihydrosphingolipids and neutral lipids are related to steatosis and fibrosis damage both in human and animal models of NAFLD

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T 06

Background:

Non-alcoholic fatty liver disease (NAFLD) is a growing entity related to the epidemic of obesity and diabetes. (Dihydro)sphingolipids are class of lipid molecules whose synthesis respond to the increased burden of circulating free fatty acids associated to NAFLD. Blocking (dihydro)sphingolipid synthesis has been proposed to overcome NAFLD. However, there are conflicting results regarding the relationship of (dihydro)sphingolipids levels and the degree of liver histological damage in patients and animal models. We use a diet induced model of NAFLD and apply lipidomics to study the relationship of lipids with NAFLD and explore which among these molecules are most related with the histological changes throughout its progression.

Methods:

C57BL/6J mice were fed a high fat diet supplemented with glucose and fructose in drinking water up to 40 weeks. A subset of animals were treated with CCl4 (ip.) for 6 and 10 weeks to induce a more advanced fibrosis stage (>F2). Animals were sacrificed at different time points to reproduce the spectrum of histological damage found in human disease: NAFL, NASH and NASH-fibrosis. The liver histology was grading following the SAF classification system (steatosis, ballooning/inflammation, fibrosis). In a complementary study, plasma and liver tissue were obtained from 200 patients whose diagnosis and NAFLD severity was evaluated by liver biopsy. Lipidomic analysis was performed using RPLC-tandem mass spectrometry following a quality control strategy to reduce potential quantification biases. The molar concentrations are reported in nmol/mg (liver) and nmol/mL (plasma).

Results:

Both animal and human liver neutral (CE and TG) concentrations were significantly increased with the steatosis burden and were downregulated by advanced fibrosis. On the contrary, (dihydro)sphingolipids concentrations were increased both by steatosis and fibrosis stage. Concentrations of CE, TG and (dihydro)sphingolipids in plasma of patients follow a similar trend to that observed in the liver.

Conclusions:

Our study demonstrate that (dihydro)sphingolipids accumulate in liver of NAFLD and correlate with the histological degree of steatosis both in mice and humans. However, the appearance of fibrosis modulates the concentration of these lipids in the liver. Of note, plasma concentrations of lipid species demonstrate a good correlation with liver findings stressing its relationship with liver metabolism.

SESSION 3
SHOTGUN LIPIDOMICS

Session Chairs: Marcus Höring, *Ilya Levental*

14:00 – 14:30 **KEYNOTE**
Update on multi-dimensional mass spectrometry-based shotgun lipidomics and its biological/biomedical applications
Xianlin Han, *Barshop Institute, UT Health, San Antonio, TX, USA*

14:30 – 14:50 **Flexibility of a mammalian lipidome — Insights from mouse lipidomics**
Christian Klose, *Lipotype GmbH, Germany*

14:50 – 15:10 **Lipidomic signatures of NAFLD progression**
Olga Vvedenskaya, *Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany; Spectroswiss, Lausanne, Switzerland*

15:10 – 15:30 **Computational stratification and subtyping on NAFLD liver lipidomics**
Tim Daniel Rose, *LipiTUM, Chair of Experimental Bioinformatics, Technical University of Munich, Germany*

15:30 – 16:00 BREAK

Flexibility of a mammalian lipidome — Insights from mouse lipidomics

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T 07

Lipidomics has become an indispensable method for the quantitative assessment oflipid metabolism in basic, clinical, and pharmaceutical research. It allows for the generation of information-dense datasets in a large variety of experimental setups and model organisms. Previous studies, mostly conducted in mice (Mus musculus), have shown a remarkable specificity of the lipid compositions of different cell types, tissues, and organs. However, a systematic analysis of the overall variation of the mouse lipidome is lacking. To fill this gap, in the present study, the effect of diet, sex, and genotype on the lipidomes of mouse tissues, organs, and bodily fluids has been investigated. Baseline quantitative lipidomes consisting of 796 individual lipidmolecules belonging to 24 lipid classes are provided for 10 different sample types. Further more, the susceptibility of lipidomes to the tested parameters is assessed, providing insights into the organ-specific lipidomic plasticity and flexibility. This dataset provides a valuable resource for basic and pharmaceutical researchers working with murine models and complements existing proteomic and transcriptomic datasets. It will inform experimental design and facilitate interpretation of lipidomic datasets.

Lipidomic signatures of NAFLD progression

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T 08

Non-alcoholic fatty liver disease (NAFLD) is a metabolic dysfunction leading to hepatic accumulation of TG in the absence of heavy alcohol consumption. However, if NAFLD effects the liver lipidome beyond flooding it with neutral lipids is poorly understood. A study cohort of 365 patients (124 and 241 m/f, respectively) included normal control group (49), patients with obesity but with histologically healthy liver (51), patients with NAFL (143), and NASH (94). Metadata included mutation status of 6 common NAFLD-related protein factors and anamnesis. High abundance of neutral (TGs and DGs) lipids compromised the lipidome coverage of FT MS analysis and therefore total extracts of liver biopsies were analyzed by the t-SIM method that increased the number of identified lipids by ca 30 %. In total, we quantify the molar abundance of 316 species from 22 major lipid classes, including membrane (GPL, SL and Chol) and neutral (DG, TG and CE) lipids. We confirmed the progressive accumulation of DG, TG and CE in the liver of NAFL and NASH patients, while the bulk composition GPL and SL did not change. To identify lipid classifiers of NAFLD we applied MoSBi biclustering. Neutral lipids, such as CE 18:3, DG 34:1, and TG 50:1 gradually increased with NAFLD onset and progression towards NASH[1]. Among membrane lipids, SM species comprising n24:0 and n24:2 fatty acid moieties showed different dynamics during NAFLD. The relative abundance of SM 43:3;2 increased during the transition from early to late NAFL, while the abundance of SM 43:1;2 decreased. Hence, they could serve as the same lipid class classifiers to differentiate reversible (early) and irreversible (late) stages of NAFL.

1 O Vvedenskaya and TD Rose et al. „Non-alcoholic fatty liver disease Stratification by Liver Lipidomics." Journal of Lipid Research (2021): 100104

Computational stratification and subtyping on NAFLD liver lipidomics

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T 09

Personalized medicine aims to offer individualized diagnoses and treatments for patients. Identification of robust disease subtypes requires multi-dimensional molecular data to infer underlying pathomechanisms and disease markers. Lipidomics provides an important additional molecular dimension, especially for investigating pathomechanisms underlying metabolic diseases. In addition, novel data science methods and AI are necessary to molecularly stratify patients and identify molecular subtypes.

We developed a biclustering ensemble method MoSBi (Molecular signature Identification from Biclustering). Biclustering approaches, in contrast to traditional clustering, link subgroups of samples with subsets of measured lipids or other biomolecules (multi-omics). This way, biclustering can reveal molecular signatures in data that traditional clustering may miss. Various biclustering algorithms have been published but they show heterogeneous performances over different datasets and many require expert knowledge and prior parameter optimization. MoSBi, however, combines the results of multiple algorithms overcoming the specifics of single algorithms while automatically optimizing computational parameters for each new dataset to yield a robust identification of subgroup-specific molecular signatures. Additionally, it offers intuitive network-based visualizations of its results.

Here, we analyzed the liver lipidome of over 350 patients at different stages of non-alcoholic fatty liver disease (NAFLD). We applied MoSBi to stratify these patients based on their liver lipidome[1]. NAFLD is a common disease in the Western society, but the links between disease progression and lipid metabolism are not yet unraveled. Non-alcoholic fatty liver (NAFL) is histologically characterized by the accumulation of triglycerides (TG) in hepatocytes. With MoSBi, we identified a strong molecular heterogeneity within the NAFL cohort: patients from the NAFL cohort exhibited high similarities in their lipid signatures to obese patients with an otherwise healthy liver on the one hand and patients diagnosed with non-alcoholic steatohepatitis (NASH) on the other hand. This suggests that there are at least two NAFL subtypes and that disease progression is indeed expressed by the lipidome even though the bulk of lipids are not showing levels of dysregulation. The most prominent signature lipids were specific odd chain sphingomyelins, whose ratios may serve

as markers for the disease status. Our lipidomics-based subtyping is also highly correlated with clinical confounders such as NAS (NAFLD Activity Score) fat and ballooning.

In summary, MoSBi is a novel approach to stratification and subtyping based on heterogeneous omics data. Here, we showed it's potential utilizing clinical lipidomics data for subtyping and biomarker detection. MoSBi is available as a web service (https://exbio.wzw.tum.de/mosbi) which researchers can utilize to apply all methods without expert knowledge and as an R package (https://gitlab.lrz.de/lipitum-projects/mosbi) for local use.

1 O Vvedenskaya and TD Rose et al. „Non-alcoholic fatty liver disease Stratification by Liver Lipidomics." Journal of Lipid Research (2021): 100104.

SESSION 4
CLINICAL LIPIDOMICS – CURRENT APPLICATIONS

Session Chairs: Irina Alecu, Kim Ekroos

16:00 – 16:30 **KEYNOTE**
Plasma ceramides as biomarkers of cerebrovascular disease and dementia
Michelle Mielke, *Mayo Clinic, Rochester, Minnesota, USA*

16:30 – 16:50 **Comprehensive lipidomic profiling along the clinical patient journey by laser assisted rapid evaporative ionisation mass spectrometry – from cancer screening to MS-guided surgery**
Zoltan Takats, *Imperial College London, United Kingdom*

16:50 – 17:10 **Altered Plasma, Urine, and Tissue Profiles of Sulfatides and Sphingomyelins in Renal Cell Carcinoma Patients**
Robert Jirásko, *Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic*

17:10 – 17:30 **Fingerprints and footprints of sebum associated with profiles of abundance of epidermal lipids in the stratum corneum: relevance in skin physiology and in atopic dermatitis.**
Emanuela Camera, *San Gallicano Dermatological Institute - IRCCS, Rome, Italy*

17:30 **PRESENTATION OF REMOTE POSTERS**

18:00 – 21:00 POSTER SESSION & BEER

Comprehensive lipidomic profiling along the clinical patient journey by laser assisted rapid evaporative ionisation mass spectrometry - from cancer screening to MS-guided surgery

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Petra Paizs, Lauren Ford, Toma Ramonaite, Yuchen Xiang, Daniel Simon, Burak Temelkuran, James Kinross

Imperial College London, United Kingdom

T 10

The versatile biochemical function of lipids and their straight forward detectability turns mass spectrometry-based lipidomics into one of the most comprehensive clinical diagnostic tools. The advent of ambient MS 15 years ago has raised the possibility of developing a universal diagnostic tool covering different clinical needs from microbiology through clinical chemistry to histopathology, however this promise has remained unfulfilled. Rapid Evaporative Ionisation Mass Spectrometry (REIMS) has originally been conceived as a method for the real-time, in-vivo identification of biological tissues in course of medical interventions. The method utilises the aerosol formed on the ablation of tissues by surgical energy devices as a sample for direct mass spectrometric analysis. REIMS can deliver tissue lipid profiles with 250 ms time delay relative to the ablation step as it was successfully demonstrated in case of human colorectal cancer resection interventions. Surgical lasers working in the mid infrared range (2-11 µm) have successfully been used in a REIMS setup and this combination was termed laser assisted REIMS (LA-REIMS) to stress the difference between this technique and laser desorption ionisation. Due to the straightforward focusability of surgical lasers, the technology was reverse translated to an MS imaging platform, capable of cellular resolution (≈10 µm feature resolution) imaging of tissue specimens. The versatility of LA-REIMS has been demonstrated in case of colorectal cancer, where the technology was used for the assessment of stool samples for screening, followed by MS-guided endoscopy, MS imaging of biopsy specimens for detailed diagnostics of dysplastic polyps and cancer, and MS-guided surgery aimed at the complete resection of tumour and assessment for the need of adjuvant therapy. The dual functionality of the mid-IR LA-REIMS setup not only bridged the histology, endoscopy and surgery applications, but also enabled the in-vivo tracking of individual cell markers. For instance, combination of SM, BMP and FA profiles were found to be informative regarding not only the spatial localisation of tumour infiltrating CD8+ T cell populations in colorectal cancer, but also found to give information about their metabolic fitness. This information was also successfully associated with the metastatic potential of tumours (ie. localised vs. metastatic disease), which in turn is critically important from surgical point of view. Cellular lipid markers were also identified for 28 different genera of commensal and pathogenic bacteria inhabiting the gastrointestinal tract and in-vivo LA-REIMS tools were successfully used to assess the composition of the colorectal mucosal microbiome including the detection of Fusobacterium spp., which were broadly associated with colorectal carcinogenesis. The colorectal cancer example clearly demonstrates that a single shotgun lipidomics tool can be used for (1) multi-step screening (stool + endoscopy), (2)establishing detailed diagnosis in a histopathology lab including the assessment of microbiome inference and (4)guiding the surgical treatment of patients.

Altered Plasma, Urine, and Tissue Profiles of Sulfatides and Sphingomyelins in Renal Cell Carcinoma Patients

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T 11

Renal cell carcinoma (RCC) represents the most common type of kidney cancer with the highest incidence and mortality rate among all urological malignancies. Biochemical processes related to RCC result in an altered lipidomic profile in body fluids that can be helpful for early cancer detection. However, the application of reliable quantitative analysis is necessary to reveal these alternations. In this work, we analyzed 599 plasma, urine, and tissue samples from 368 of RCC patients and healthy controls. Mass spectrometry-based profiling of sulfatides and sphingomyelins in plasma and urine samples shows appreciable concentration changes in renal cell carcinoma patients compared with healthy controls. Elevated concentrations of lactosylsulfatides, decreased concentration level of sphingomyelines with long saturated N-fatty acyls and sulfatides with hydroxylated fatty acyls show to be the most important dysregulations in cancer. The positive correlation between the results obtained for body fluids and tissues indicates that the altered sphingolipid profile is closely associated with tumor progression. This work was supported by the Czech Science Foundation (project No. 21 20238S).

Fingerprints and footprints of sebum associated with profiles of abundance of epidermal lipids in the stratum corneum: relevance in skin physiology and in atopic dermatitis.

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T 12

Sebum is an oily to waxy secretion produced by the sebaceous gland (SG). Growing evidence support the participation of sebum in the plasticity of the skin permeability barrier (SPB) in the stratum corneum (SC), wherein ceramides, long chain free fatty acids, and cholesterol metabolites serve both structural and biophysical functions. Derangement of the SPB plays a primary role in the pathogenesis of atopic dermatitis (AD), which is a chronic and recurrent inflammatory skin disorder characterized by xerosis and itch. Although scarcely investigated, impaired sebum production has been proposed as a key feature in AD. By integrating TLC, GCMS and high-resolution LCMS in the analytical workflow, we quantified the molar abundance of about 300 species from 8 major lipid classes present in the two lipid matrices, i.e. sebum and the SPB, sampled at the skin surface of 44 adult healthy controls (hC) and 54 AD patients of both genders. The AD patients included 20 cases with normal sebum secretion and clinical manifestations absent on the face (fnAD). The remaining 34 cases presented hyposeborrhea and clinical manifestations extended to the face (fyAD). Fingerprints of sebaceous lipids were acquired by TLC and GCMS in sebum sampled from SG-rich sites by tape absorption. Footprints of SG-activity and profiles of abundance of epidermal lipids were determined by GCMS and LCMS, respectively, in the SC sampled from SG-rich and SG-poor sites. The results demonstrated significant depletion of sebum-specific lipids in AD, more pronounced in fyAD than fnAD. Sebum footprints were weaker in the SC of AD, regardless the site. The significant differences found between males and females of the hC group, observed in both fingerprints and footprints of sebum, disappeared in the AD group. The profiles of abundance of epidermal lipids were significantly different between SG-rich and SG-poor areas in both hC and AD groups. CerNP and CerNH had lower levels, whereas CerAS, CerAP, and CerAH had higher levels in SG-rich areas. The levels of CerNP and CerNH were significantly lower in non-sebaceous areas of AD patients compared to hC. Nevertheless, the depletion was milder when sapienate, a sebum specific MUFA, was more abundant. Comparison of profiles of abundance of ceramides between hC and AD showed that the pathogenic elevation of short chain ceramides, especially CerNS with 34-36 carbon-atoms, characteristic of AD's SPB, was more pronounced in the SG-rich areas. Globally, linoleoyl-hydroxyceramides (C18:2-CerEO) presented comparable levels in SG-rich and SG-poor areas. Nevertheless, C18:2-CerEO were significantly depleted in AD, independently on the SG-density. In conclusion, the data indicate that (i) the SG activity participates in the SBP integrity; (ii) the sebum secretion partly compensates for the SPB perturbation in AD; (iii) the impairment of the SG secretion associates with AD severity and exacerbates the AD-like ceramides' derangement.

SESSION 5
STRUCTURAL LIPIDOMICS

Session Chairs: Kai Schuhmann, Michal Holčapek

9:00 – 9:30 **KEYNOTE**
Integrating Hydrophilic Interaction Chromatography, Trapped Ion Mobility, and Isomer Resolving MS/MS Enables Fast and In-Depth Lipidomic Profiling
Yu Xia, *Department of Chemistry, Tsinghua University, Beijing, China*

9:30 – 9:50 **Mapping of alkyl and alkenyl ether lipids in RP-LC-MS experiments**
Jakob Koch, *Institute of Human Genetics, Medical University of Innsbruck, Austria*

9:50 – 10:10 **HILIC meets MS imaging: CCS values obtained by chromatographic separation coupled to trapped ion mobility-MS for unequivocal assignment of phospholipids in MALDI-MS-imaging**
Ansgar Korf, *Bruker Daltonics GmbH & Co. KG, Bremen, Germany*

10:10 – 10:30 **Identification and quantitation of glycolipidicrhamnolipids by supercritical fluid chromatography-mass spectrometry and charged aerosol detection**
Anna Lipphardt, *Institute of Inorganic and Analytical Chemistry, University of Münster, Münster, Germany*

10:30 – 11:00 BREAK

Mapping of alkyl and alkenyl ether lipids in RP-LC-MS experiments

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T 13

Cellular membranes are comprised of a large diversity of phospholipid species that take on structural, but also metabolic as well as signaling functions. Additionally to the specific head groups, phospholipids can be further characterized according to their exact radyl side-chain substitution. Beside the well-known esterification with acyl residues, the so-called ether lipids are substituted with alkyl or alkenyl residues at the sn-1 position, and account for up to 20% of the total phospholipid content. Ether lipids are not only structurally but also functionally distinct, which can be seen, for example, in the bioactive function of one of their representatives, the platelet-activating factor, or by the severe symptoms of inherited diseases that impair their biosynthesis such as rhizomelic chondrodysplasia punctate.

Despite their abundance and physiological importance, ether lipids are often neglected in lipidomics experiments, especially when it comes to distinguishing between plasmalogens (alkenyl linkage) and other ether lipids (alkyl linkage). Reasons for this are that an exact distinction is difficult due to the many isobaric possibilities and that only insufficient commercial standards are available. One illustrative example is the pair PE(O-18:1/22:4) and PE(P-18:0/22:4) with identical mass and sum formula (753.567242 Da, $C_{43}H_{80}NO_7P$). Even with high-resolution tandem mass spectrometry these two compounds cannot be distinguished, as the ether/vinyl-ether linked *sn*-1 fragments ionize poorly in standard collision-induced dissociation experiments, resulting in identical MS^2 spectra.

Historically, a variety of different biochemical assays were developed which were able to distinguish utilizing differential analysis in combination with derivatization or saponification strategies, and laborious sample preparation procedures. Recently, a plasmalogen-specific labeling assay was developed which allowed assigning the gene encoding for the PEDS (plasmanylethanolamine desaturase) enzyme that forms plasmalogen from ether lipid substrates. Subsequently, this permitted us to compare a broad range of tissue lipidomes of plasmalogen deficient mice with mainly plasmalogen containing wild types. Characterizing the relative retention time effect of isomeric alkyl/alkenyl-pairs enabled us to reliably chromatographically separate these two important ether lipid subclasses and to describe a generalizable behavior that can now be implemented in standard lipidomics workflows. This will also be of great benefit when developing and validating new mass spectrometric approaches relying on the rapidly improving instrument capabilities for the consistent differentiation between different ether lipid categories.

HILIC meets MS imaging: CCS values obtained by chromatographic separation coupled to trapped ion mobility-MS for unequivocal assignment of phospholipids in MALDI-MS-imaging

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T 14

The investigation of lipids is of paramount importance due to their involvement in various biological and physiological processes. An important lipid category are glycerophospholipids with a variety of different head groups in combination with aliphatic chains with different numbers of carbon atoms and double bonds. High-resolution mass spectrometry is capable of identifying lipid species in combination with fragmentation experiments (MS/MS) based on the accurate m/z and fragmentation pattern. For the differentiation of isomeric lipids or isobaric interferences, however, powerful separation methods are required. Especially in imaging techniques, such as MALDI-MS imaging, the identification usually relies exclusively on the exact m/z. In recent years, ion mobility spectrometry, especially in combination with MS, has become a highly relevant topic in current lipid research. The use of trapped ion mobility-mass spectrometry (TIMS) for separation of ions after MALDI ionization increases the reliability identification based on a lipid's collision cross section (CCS).

However, the availability of CCS libraries is a major bottleneck. To overcome this issue, we created a tissue specific CCS database using hydrophilic interaction liquid chromatography coupled to TIMS-MS. With the help of this database we were able to clearly distinguish isomeric lipid classes such as phosphatidylglycerol and its regio-isomer bis (monoacylgylcero) phosphate as well as isobaric interferences due to adduct formation during the MALDI process. The developed workflow was successfully applied to a mouse spleen sample for an unequivocal lipid identification applying MALDI-TIMS-MS imaging.

Identification and quantitation of glycolipidic rhamnolipids by supercritical fluid chromatography-mass spectrometry and charged aerosol detection

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Heiko Hayen

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T 15

The class of glycolipidic rhamnolipids, which are composed of one or two L-rhamnose units and up to three β-hydroxy fatty acids, are mainly known for their application as a bio-based alternative to synthetic surfactants. However, there are other applications of rhamnolipids that are no less important. One example is their antibiofilm activity. Although biofilms are extremely difficult to remove because the cells are cemented in a polymer matrix, rhamnolipids, purified or in bacterial supernatants (e. g. *Pseudomonas aeruginosa*), can disperse biofilms and, in the case of sulfate-reducing bacteria consequently reduce metal corrosion.[1] Rhamnolipids have also been shown to have antimicrobial effects, including in healthy skin[2] and against oral pathogens[3]. In this context, the use of rhamnolipids represents an opportunity for alternative antimicrobial strategies and oral-related applications and should be further explored for the benefit of global health.[4]

In this work, analytical methods for the identification, structural characterization, and quantification of rhamnolipids were developed. Since in recent years, sustainability has become increasingly important in science and in public awareness, methods based on SFC instead of HPLC were chosen with the aim of creating the basis for a resource-saving analytical technique. The reasons behind are the high efficiency of the separation as well as savings in solvent consumption, as the main part of the mobile phase is replaced by supercritical carbon dioxide for SFC.

Herein, we present a hyphenation of SFC with MS for rapid identification of rhamnolipids. In particular, SFC provides fast separation of rhamnolipid subclasses, which differ in the number of L-rhamnose units and linked β-hydroxy fatty acids, and the differentiation of rhamnolipids from their precursors, 3-(3-hydroxyalkanoyloxy)alkanoic acids (HAA). Applying this method, 33 species were identified in a fermentation supernatant of *Pseudomonas putida* strainKT2440 based on accurate mass and data dependent fragmentation, with species with one or two β-hydroxydecanoic acid chains dominating. Furthermore, due to its universal detector response, coupling with charged aerosol detection (CAD) enabled the complementary quantification of the absolute contents of the individual subclasses using a structurally related standard compound.

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2 U. Meyer-Hoffert, A. Zimmermann, M. Czapp, J. Bartels, Y. Koblyakova, PLoS One2011, 6, 16433.
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4 J. Chen, Q. Wu, Y. Hua, J. Chen, H. Zhang, H. Wang, Appl. Microbiol. Biotechnol. 2017, 101, 8309–8319

SESSION 6
SPHINGOLIPIDS SIGNALING/HEALTH AND DISEASE

Session Chairs: Stefanie Rubenzucker, Gerhard Liebisch

11:00 – 11:30 **KEYNOTE**
Ceramides and the two phases of lipotoxicity?
Scott Summers, *University of Utah, Salt Lake City, Utah, USA*

11:30 – 11:50 **A REFERENCE MAP OF SPHINGOLIPIDS IN MURINE TISSUES**
Sneha Muralidharan, *Singapore Lipidomics Incubator, Life Sciences Institute, National University of Singapore, Singapore*

11:50 – 12:10 **Probing the platelet lipidome and identifying key lipids critical for platelet activation by comprehensive lipidomics**
Robert Ahrends, *Department of Analytical Chemistry, University of Vienna, Vienna, Austria*

12:10 – 12:30 **Gangliosides Characterization and Isomer Separation using SLIM-based High Resolution Ion Mobility (HRIM)-Mass Spectrometry (MS)**
Komal Kedia, *MSD, West Point, PA 19486 USA*

12:30 – 14:00 LUNCH

13:30 – 14:00 **LUNCH WORKSHOP**
Lipid Characterization with EAD using the ZenoTOF 7600 system
Christian Baumann, *Accurate Mass Workflows, SCIEX*

A reference map of sphingolipids in murine tissues

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Mitsugu Shimobayashi[2], Bo Burla[1], Michael N. Hall[2], Markus R. Wenk[1], Federico Torta[1]

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T 16

Sphingolipids (SP) have both a structural role in the cell membranes and a signaling function that regulates many cellular processes. The enormous structural diversity and low abundance of many SP pose a challenge for their identification and quantification. Recent advances in lipidomics, in particular liquid chromatography (LC) coupled with mass spectrometry (MS), provide methods to detect and quantify many low abundant SP species reliably. Here we use LC-MS to compile a ‚murine sphingolipid atlas', containing the qualitative and quantitative distribution of 114 SP in 21 tissues of a widely utilized wild-type laboratory mouse strain (C57BL/6). We report tissue-specific SP fingerprints, as well as sex-specific differences in the same tissue. This is a comprehensive, quantitative sphingolipidomic map of mammalian tissues collected in a systematic fashion. It will complement other tissue compendia for interrogation into the role of SP in mammalian health and disease.

Probing the platelet lipidome and identifying key lipids critical for platelet activation by comprehensive lipidomics

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Cristina Coman[1], Mailin-Christin Manke[2], Bing Peng[3], Dominik Kopczyinski[1], Stefanie Rubenzucker[1], Nils Hoffmann[4], Patrick Münzer[2], Meinrad Gawaz[2], Oliver Borst[2]

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T 17

Platelet integrity and function critically depend on lipid composition. However, the lipid inventory in platelets was hitherto not quantified. So far, 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 600 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. Using different model systems, we were able show the adaptation of the platelet lipidomes in diseases and diet and a very specific modulation of the platelet lipids with an order of magnitude upregulation of signaling but also membrane components such as sphingomyelin. As signaling lipid we identify lysosphingomyelin and demonstrated its direct impact on platelet activation and thrombus formation. In conclusion, our ongoing quantitative lipidomics research in platelets sheds light on novel mechanisms important for platelet function, and has therefore the potential to open novel diagnostic and therapeutic opportunities.

Gangliosides Characterization and Isomer Separation using SLIM-based High Resolution Ion Mobility (HRIM)-Mass Spectrometry (MS)

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T 18

Gangliosides (GG) are a large family of glycosphingolipids containing acidic mono/poly- sialylated glycans attached to a ceramide backbone. Accumulation of GGs have been implicated in several neurodegenerative disorders including Alzheimer's and Parkinson's. GGs are a highly diverse class of compounds due to variable chain lengths of the ceramide backbone in combination with variable uncharged sugar moieties. Due to this structural heterogeneity, gangliosides analysis remains challenging using traditional LC-MS based platforms.

Structures for Lossless Ion Manipulation (SLIM), has shown promising results in resolving molecules isobaric or isomeric in nature that typically co-elute in LC dimension, with unprecedented IM resolution and faster analysis times. Here we report a high throughput LC-HRIM-MS method for faster, more in-depth characterization of gangliosides in biological matrices.

GGs were extracted from pure standards and mouse brain homogenate using liquid-liquid extraction by addition of chloroform/methanol followed by mixing and centrifugation. The upper aqueous phase containing gangliosides was collected and further concentrated using a C-18 SPE. Eluates were dried and reconstituted in chloroform/methanol just prior to analysis. Data was collected on the HRIM device, coupled to an Agilent 6545XT QTOF in both FIA and LC modes on a HILIC column (two gradients). Data processing was accomplished using MOBILion's HRIM Data-Processor and Agilent's IM-MS Browser.

Our initial goal was to investigate the coverage and response of different gangliosides on the HRIM platform comparing three different modes: 1) flow injection analysis, 2) HILIC LC – 5min separation time, and 3) HILIC LC – 10 min separation time. First, we ran neat standards of individual gangliosides mainly, GM1, GD1a, GD1b, GT1b, GQ1b and a deuterated GM3 to determine their unique arrival times, followed by running extracted mice brain homogenate to explore endogenous gangliosides. All these samples were run in the three modes mentioned above. Multiple gangliosides species were seen in all three modes covering major classes: GM1, GD1a, GD1b, GT1b and GQ1b in mouse brain extract. As expected, incorporating a LC separation step prior to HRIM mitigates ion suppression from abundant species and produced enhanced response as compared to FIA only mode. With both LC methods we observed multiple potential isomers of several gangliosides including, but not limited to GD1: m/z 917.47 (-2) - 4 isoforms, m/z 1043 – 3 isoforms; GT1b: m/z 1063 (-2) - 3 isoforms, m/z 1074 (-2) – 3 isoforms. These preliminary findings are encouraging and currently method validation to determine LLOD, LLOQ, linearity, accuracy and precision is underway.

SESSION 7
BILE ACIDS AND LIPID SIGNALING

Session Chairs: Bing Peng, Justine Bertrand-Michel

14:00 – 14:30 **KEYNOTE**
Bile acid signaling and the regulation of metabolic control
Christian Wolfrum, *Institute of Food Nutrition and Health, ETH Zürich, Zürich, Switzerland*

14:30 – 14:50 **Short chain fatty acids and bile acids in human faeces are associated with the intestinal cholesterol conversion status**
Silke Matysik, *Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany*

14:50 – 15:10 **CRISPR/Cas-9-mediated inactivation of soluble epoxide hydrolase N-Tle domain, a lipid phosphate phosphatase, promotes thermogenesis through peroxisome proliferator activated receptor gamma.**
Thomas Duflot, *Normandy University, UniRouen, Inserm UMR1096 EnVI, Rouen, France;Department of Pharmacology, Rouen University Hospital, Rouen, France; Laboratory of Pharmacokinetics, Toxicology and Pharmacogenetics, Rouen University Hospital, Rouen, France*

15:10 – 15:30 **Assessing membrane fluidity of antibiotic-resistant Staphylococcus aureus using an RPLC-IM-MS method for isomeric phospholipid separations**
Kelly M. Hines, *Department of Chemistry, University of Georgia, Athens, GA, United States of America*

15:30 – 16:00 BREAK

Short chain fatty acids and bile acids in human faeces are associated with the intestinal cholesterol conversion status

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T 19

The analysis of human faecal metabolites can provide an insight into metabolic interactions between gut microbiota and host organism. The creation of metabolic profiles in faeces has received little attention until now and reference values, especially in the context of dietary and therapeutic interventions, are missing. The aim of the present study is to give concomitant concentration ranges of faecal sterol species, bile acids and short chain fatty acids based on a large cohort.

Sterol species, bile acids and short chain fatty acids in human faeces from 165 study participants were quantified by LC-MS/MS. For standardization, we refer all values to dry weight of faeces. Based on the individual intestinal sterol conversion we classified participants into low and high converters according to their coprostanol/cholesterol ratio.

Based on a large number of study participants we give a general quantitative overview of several metabolites in human faeces that can be used as reference values. The intestinal cholesterol conversion is a distinctive feature to evaluate SCFA and bile acid concentrations. Patient stratification into high or low sterol converter groups is associated with significant differences in faecal metabolites with biological activities. Such stratification should then allow assessing faecal metabolites better before therapeutic interventions. Low converters excrete significantly more straight chain fatty acids and bile acids than high converters. 5th, 95th percentile and median of bile acids and short chain fatty acids were calculated for both groups.

The strength of our calculation is that our data base on i) a large cohort, ii) an uncontrolled diet which should reflect the behaviour of the normal population and iii) a comprehensive data set from various countries in Europe.

CRISPR/Cas-9-mediated inactivation of soluble epoxide hydrolase N-Tle domain, a lipid phosphate phosphatase, promotes thermogenesis through peroxisome proliferator activated receptor gamma.

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T 20

The physiological role of the C-terminal hydrolase domain of the soluble epoxide hydrolase (sEH-H), which metabolizes epoxyfatty acids to diols is well investigated. However, the function of its N-terminal phosphatase activity (sEH-P) remains unclear although in vitro data suggest that it dephosphorylates intracellular lysophosphatidic acids (LPA) to monoacyglycerols (MAG). CRISPR/Cas9 was used to generate a novel knock-in (KI) rat line lacking the sEH-P activity. Plasma levels of sEH substrates and metabolites for both the C- and N- terminal domains were determined by tandem mass spectrometry and plasma lipidome was assessed by untargeted analysis. Thermogenesis was evaluated using cold tolerance test in the presence or absence of an antagonist of peroxisome proliferator activated receptor gamma (PPARγ), which is the only intracellular receptor activated by LPA. In addition, a transcriptomic analysis was performed on brown adipose tissue using DNA microarray. Body composition was measured by echoMRI and glucose and insulin tolerance tests were performed in rats fed a normal or a high-fat diet. sEH-P KI rats exhibited a decreased metabolism of LPA to MAG without change in diol-to-epoxyde ratios. Lipidomic analysis revealed an increase in several phosphatidylcholine species. The sEH-P KI rats grew almost normally but with less weight and fat mass gain while insulin sensitivity was increased compared to wild-type rats. This lean phenotype was more marked in males than in female KI rats. In fact, transcriptomic analysis revealed that sEH-P KI rats had an increased lipolysis allowing them to supply fatty acids as fuel to potentiate brown adipose thermogenesis under resting condition and upon cold exposure. The potentiation of thermogenesis in sEH-P KI rats was abolished when blocking PPARγ, but also when inhibiting simultaneously sEH-H, showing a functional interaction between the two domains. Furthermore, sEH-P KI rats fed a high-fat diet did not gain as much weight as the wild-type rats, did not have increased fat mass and did not develop insulin resistance or hepatic steatosis. Our study thus reveals that sEH-P is a key player in energy and fat metabolism and contributes together with sEH-H to the regulation of metabolic homeostasis.

Assessing membrane fluidity of antibiotic-resistant Staphylococcus aureus using an RPLC-IM-MS method for isomeric phospholipid separations

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T 21

Introduction:

Staphylococcus aureus varies its membrane fluidity in response to environmental stresses by changing the ratio of branched-chain fatty acids (BCFAs) to straight-chain fatty acids (SCFAs) in its membrane lipids. Altered membrane fluidity has been associated with an increased tolerance of membrane-targeting antibiotics, including daptomycin. The routine assessment of microbial membrane fluidity relies on the measurement of total BCFA-versus-SCFA determination by GC-MS. Although GC-MS is capable of resolving BCFA and SCFA isomers, the requirement of free fatty acids eliminates the possibility to evaluate the preferences of lipid subclasses for BCFAs vs. SCFAs. We recently demonstrated a RPLC method that can separate lipid isomers having branched-branched, branched-straight, or straight-straight fatty acyl tail combinations. We used this method to examine the distribution of FA isomers in the lipids of a *S. aureus* strain with daptomycin resistance.

Methods:

Methicillin-resistant (N315) and daptomycin-resistant (N315-Dap8) *S. aureuswere* grown for 24 hrs and underwent lipid extraction using a modified Bligh and Dyer method. Daptomycin resistance in strain N315-Dap8 was conferred by mutations affecting eight cellular functions, including *pgsA* and *mprF*, which are responsible for phosphatidylglycerol (PG) and LysylPG biosynthesis, respectively. RPLC-IM-MS analysis was performed on a Waters UPLC connected to a Synapt XS TWIM-MS. Lipids were separated on a Waters CSH C18 column with a 30-minute gradient. Data was collected in positive and negative mode. Raw data was processed with Progenesis QI and Skyline. The Skyline method utilized a transition list of PGs and LysylPGs and a drift time library for IM filtering of coeluting phospholipid classes.

Preliminary Data:

Comparative lipidomics between *S. aureus* strains with and without daptomycin resistance revealed changes in both the absolute magnitude of lipid abundances and the distribution of FA isomers. As previously reported, N315-Dap8 had decreased levels of PGs and increased lysylPGs compared to N315 as a result of the *pgsA* and *mrpF* mutations. However, the RPLC method revealed that the PGs present in N315-Dap8 contained only branched-branched and branched-straight FA combinations, whereas N315 contained PGs with all three combinations of FAs. PGs with two straight-chain FAs appeared in N315-Dap8

when bacteria were cultured in broth containing SCFAs, but the extent of incorporation was substantially lower than observed for N315. However, LysylPGs lacked straight-straight isomers in both N315 and N315-Dap8 even when straight FAs were provided in the culture media. These results indicate that daptomycin resistance is facilitated in-part by increased membrane fluidity. Future experiments will include metabolomics analyses and measurements of membrane fluidity in order to correlate the lipidomic measurements with levels of BCFA precursors and membrane physiology, respectively.

SESSION 8
LIPID BIOMARKER

Session Chairs: Olga Vvedenskaya, Zoltan Takats

16:00 – 16:30 **KEYNOTE**
Lipidomic-Based Insights into Disease Mechanisms:
Targeted Lipidomics Discovery Pipeline for Network Analysis
Irina Alecu, *University of Ottawa, Ottawa, Ontario, Canada*

16:30 – 16:50 **Multiomics of synaptic junctions reveals altered lipid metabolism and signaling upon environmental enrichment**
Cristina Coman, *Department of Analytical Chemistry, University of Vienna, 1090 Wien, Austria*

16:50 – 17:10 **Modified lipids and lipid-protein adducts as pro-inflammatory markers in obesity and related complications**
Patricia Prabutzki, *Leipzig University, Faculty of Medicine, Institute for Medical Physics and Biophysics, Leipzig, Germany*

17:10 – 17:30 **EARLY CAREER FEMALES IN LIPIDOMICS CATEGORY**
Chemical cartography of localized lipidomic alterations during infectious diseases
Laura-Isobel McCall, *Department of Chemistry and Biochemistry, University of Oklahoma, USA*

19:30 – 23:00 CONFERENCE DINNER

Multiomics of synaptic junctions reveals altered lipid metabolism and signaling upon environmental enrichment

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T 22

Membrane lipids and their metabolism are known to have key functions in neurotransmission. Here we provide the first quantitative lipid inventory of rat synaptic junctions. To this end we used a novel multiomics extraction and analysis workflow to probe the interplay of proteins and lipids in synaptic signal transduction from the same sample. Based on this workflow we generated hypotheses about novel mechanisms underlying complex changes in synaptic connectivity elicited by environmental stimuli. As a proof of principle, this approach revealed that in mice exposed to an enriched environment reduced endocannabinoid synthesis and signaling is linked to increased surface expression of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) in a subset of Cannabinoid-receptor 1 positive synapses. This mechanism regulates synaptic strength in an input-specific manner. All in all, we established a compartment specific multiomics workflow that is suitable to extract information from complex lipid and protein networks involved in synaptic function and plasticity.

Modified lipids and lipid-protein adducts as pro-inflammatory markers in obesity and related complications

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T 23

Much of the most prevalent causes of deaths in highly industrialized countries are diseases characterized by an underlying state of chronic inflammation, such as ischemic heart disease, specific types of cancers, type 2 diabetes and related cardiovascular disorders.

Type 2 diabetes and cardiovascular diseases are often associated with obesity. This issue is of particular concern, since the prevalence of obesity is increasing steadily worldwide – not only in industrialized countries.

Obesity is characterized by the accumulation of excessive body fat predominantly in the form of lipid droplets in adipose tissue, which is accompanied by low-level chronic inflammation and redox imbalance. Dysregulation of the lipid metabolic pathways in the presence of increased numbers of reactive oxygen species, due to the redox imbalance, result in the generation of lipid peroxidation products (LPPs) such as truncated carbonyl compounds or hydroperoxides.

LPPs can act as secondary messengers in signal transduction pathways and/or form covalently bound adducts with thiol- and amino groups of proteins, acting as immune-stimulatory damage-associated molecular patterns (DAMPs).

Identification of specific redox-derived DAMPs and quantification of the LPP adducts on proteins might be useful for the evaluation of their significance as pro-inflammatory triggers in chronic inflammatory diseases.

For the investigation of this highly diverse group of oxidized lipids and their potential adducts, a combination of state of the art lipidomic analysis and immunological methods is required.

To understand the role of LPPs as well as lipid-derived protein modifications and particularly their pro-inflammatory potential, we performed analysis of reactive LPPs protein targets in different biological samples, including adipose tissue, liver and plasma. Using a combination of Dot Blot, Western blot and Liquid Chromatography Mass Spectrometry (LC-MS) analysis, LPP-derived DAMPs were identified and quantified. Furthermore, significant differences between different types of adipose tissue (subcutaneous vs visceral) and metabolic states (insulin sensitive vs insulin resistant) were demonstrated.

Analysis of the complex interplay between oxidized lipids and their protein targets will assist the discovery of early disease biomarkers and key molecules for the design of targeted intervention strategies.

Chemical cartography of localized lipidomic alterations during infectious diseases

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Department of Chemistry and Biochemistry, University of Oklahoma, USA

T 24

Spatial context is essential to understand host-microbe-environment interactions and link chemical structure to biological function. Using an approach called „chemical cartography", we combine liquid chromatography tandem mass spectrometry (LC-MS/MS), 3D modeling and data analytics with infection biology, to identify the spatial distribution of lipids and how they relate to infectious disease pathogenesis. Using this approach, we revealed how infection with Leishmania major and Trypanosoma cruzi parasites perturb lipid metabolism in a site-specific manner. Leishmania major is a cause of cutaneous leishmaniasis, a disfiguring skin condition affecting 12 million people worldwide. Chemical cartography analysis of experimental cutaneous leishmaniasis lesions revealed increases in glycerophosphocholines at the lesion site, as well as increases in specific phosphatidylethanolamines and sphingomyelins. Strikingly, several glycerophosphocholines were selectively elevated at lesion-adjacent, macroscopically-healthy sites, something that would have been missed in the absence of a spatial perspective. Trypanosoma cruzi are the causative agents of Chagas disease and infect over 6 million people worldwide. Chagas disease symptoms include cardiac arrhythmias, cardiac apical aneurysms, heart failure, enlargement of the oesophagus (megaoesophagus) and enlargement of the colon (megacolon). Chemical cartography revealed that acute T. cruzi infection was associated with increased glycerophosphocholine family members at sites of Chagas disease, including oesophagus and colon, while glycerophosphocholines were decreased by infection in the small intestine. These perturbations persisted in the oesophagus in the chronic stage of disease and were also observed in the heart, particularly at the heart apex. In contrast, chronic T. cruzi infection depleted cardiac acylcarnitines. Remedying these disturbances using carnitine administration prevented acute-stage Chagas disease mortality in an experimental mouse model without affecting parasite load, demonstrating a novel role for acylcarnitine metabolism in Chagas disease tolerance. Overall, these findings demonstrate the utility of our spatial lipidomic analyses in understanding infectious disease pathogenesis and to guide drug development.

SESSION 9
STANDARDS AND TOOLS FOR LIPID DATA PROCESSING & ANALYSIS

Session Chairs: Tim Daniel Rose, Robert Ahrends

9:00 – 9:30 **KEYNOTE**
Lipid molecular timeline profiling reveals diurnal crosstalk between the liver and circulation
Christer Ejsing, University of Southern Denmark, Odense, Denmark & EMBL, Heidelberg, Germany

9:30 – 9:50 **Lipid Creator workbench to probe the lipidomic landscape**
Dominik Kopczynski, Department of Analytical Chemistry, University of Vienna, Austria

9:50 – 10:10 **Integration of lipid specific fragmentation rules into SIRIUS**
Kai Dührkop, *Department of Bioinformatics, Faculty of Mathematics and Computer Science, Friedrich Schiller University, Jena, Germany*

10:10 – 10:30 **Smart Peak Automates Metabolomics, Fluxomic, and Lipidomic Data Processing**
Douglas McCloskey, *Technical University of Denmark, Denmark*

10:30 – 11:00 BREAK

LipidCreator workbench to probe the lipidomic landscape

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Bing Peng[1], Brian S. Pratt[3], Christer S. Ejsing[4,5], Bo Burla[6], Martin Hermansson[7], Peter I. Benke[8], Sock Hwee Tan[9,10], Mark Y. Chan[9,10,11], Federico Torta[8], Dominik Schwudke[12,13,14], Sven Meckelmann[15], Cristina Coman[2], Oliver J. Schmitz[15], Brendan MacLean[3], Mailin-Christin Manke[16], Oliver Borst[16], Markus R. Wenk[6,8], Nils Hoffmann[17], Robert Ahrends[2]

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T 25

Mass spectrometry (MS)-based targeted lipidomics enables the robust quantification of selected lipids under various biological conditions but comprehensive software tools to support such analyses are lacking. Here we present LipidCreator, a software that fully supports targeted lipidomics assay development. LipidCreator offers a comprehensive framework to compute MS/MS fragment masses for over 60 lipid classes. LipidCreator provides all functionalities needed to define fragments, manage stable isotope labeling, optimize collision energy and generate in silico spectral libraries. We validate LipidCreator assays computationally and analytically and prove that it is capable to generate large targeted experiments to analyze blood and to dissect lipid-signaling pathways such as in human platelets.

Integration of lipid specific fragmentation rules into SIRIUS

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T 26

SIRIUS[1] is a software framework for the analysis of tandem mass spectrometry data. It integrates high-resolution isotope pattern analysis and fragmentation tree computation for the task of molecular formula identification as well as several supervised machine learning methods for the prediction of molecular fingerprints, the annotation of molecular structures, and the prediction of compound classes.

However, SIRIUS was designed for the analysis of small metabolites for which no simple fragmentation rules are known. For compound classes with well described fragmentation behaviour, such as peptides or certain lipid classes, SIRIUS might perform worse than tools specialized for these specific classes. In particular, lipids with high masses that generate only few fragment peaks are difficult to annotate with the use of fragmentation trees.

We updated the SIRIUS algorithm and improved its performance on lipids: We implemented fragmentation rules for glycerolipids, glycerophospholipids, and sphingolipids, as well as their subclasses. Given a tandem mass spectrum of an unknown analyte, SIRIUS first checks whether the ion mass could correspond to a lipid and annotates the peaks in the spectrum with fragments and losses specific for one of the lipid classes mentioned above. It also detects peaks that can be explained as fragments or losses of fatty acids or alkyl chains. A linear support vector machine is trained to decide whether the annotated spectrum is indeed a lipid; we use features like the presence of certain fragments and losses, the explained intensity, and the number of annotated peaks. For spectra which are classified as lipid, the peak annotations are passed to the SIRIUS algorithm which then annotates the remaining peaks in the spectrum. Since some peaks are already annotated, the computation of the fragmentation tree takes considerably less time and is completed in less than a second in almost all cases. If the spectrum is not classified as lipid, the standard SIRIUS fragmentation tree algorithm is used. We improved the SIRIUS scoring by adding typical lipid losses and fragments to the set of common fragments and losses. Furthermore, losses with the molecular formula of a fatty acid chain are rewarded.

By combining a fast and simple algorithm with strict fragmentation rules with the flexible SIRIUS fragmentation tree algorithm for general small molecules, we can analyze most lipid spectra, but are still able to identify lipids that either do not fit into any of these simple lipid classes, show very different fragmentation behavior, or are modified in some way (e.g., halogenated lipids). This makes SIRIUS an excellent choice for analyzing datasets containing potentially unknown lipids and a mixture of different compound classes.

1 Dührkop, Kai, et al. „SIRIUS 4: a rapid tool for turning tandem mass spectra into metabolite structure information." Nature methods 16.4 (2019): 299-302

SmartPeak Automates Metabolomics, Fluxomic, and Lipidomic Data Processing

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T 27

Technological advances in high-resolution mass spectrometry (MS) vastly increased the number of samples that can be processed in a life science experiment, as well as volume and complexity of the generated data. To address the bottleneck of high-throughput data processing, we present SmartPeak (https://github.com/AutoFlowResearch/SmartPeak), an application that encapsulates advanced algorithms to enable fast, accurate, and automated processing of capillary electrophoresis-, gas chromatography-, and liquid chromatography (LC)–MS(/MS) data and high-pressure LC data for metabolomics, lipidomics, and fluxomics experiments. The application allows for an approximate 100-fold reduction in the data processing time compared to manual processing while enhancing quality and reproducibility of the results.

Recent work in applying SmartPeak to address the challenges of designing organisms in synthetic biology and identifying novel natural products will be discussed.

SESSION 10
DATA INTEGRATION

Session Chairs: Dominik Kopczynski, Dominik Schwudke

11:00 – 11:30 **KEYNOTE**
LION/web: a web-suite for lipidomics data analysis
Martijn Molenaar, *Alexandrov Group, EMBL, Heidelberg, Germany*

11:30 – 11:50 **Automation of Large-Scale Lipidomics Workflow Management, Data Processing, QA/QC and Reporting**
Bo Burla, *National University of Singapore, Singapore*

11:50 – 12:10 **The LIFS tools and workflows for mass-spectrometry based lipidomics**
Nils Hoffmann, *Center for Biotechnology (CeBiTec), Bielefeld University, Germany*

12:10 – 12:30 **Investigating Global Lipidome Alterations with the Lipid Network Explorer**
Nikolai Köhler, *LipiTUM, Chair of Experimental Bioinformatics, TUM School of Life Sciences, Technical University of Munich, Freising, Germany*

12:30 – 12:45 **AWARD CEREMONY**
Poster and Presentation Prizes

12:45 – 13:00 **Presentation of ILS 2022**
Session Chair: Erin S. Baker

13:00 – 14:00 LUNCH AND DEPARTURE

Automation of Large-Scale Lipidomics Workflow Management, Data Processing, QA/QC and Reporting

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T 28

Data automation in the management of analytical workflows, QA/QC, data processing, and reporting is a key factor in conducting large-scale and complex bioanalytical projects.

We have implemented an enterprise-grade Laboratory Information Management System (LIMS) to enable standardized reproducible and scalable management of projects, analytical workflows, samples and instruments. We are currenrtly exploring different approaches toward a more efficient MRM raw data processing, which is still a major challenge and bottleneck for large-scale assays. Futhermore, we developed a software pipeline (MIDAQr) for the automated yet supervised data post-processing, QA/QC and reporting of pre-processed lipidomics raw data. This tool distinguishes itself from other available lipidomics tools by being focus on data processing, more flexible in handling the diversiy of analytical methods and data processing strategies and in providing specific QC information to improve data. The output of this tool in form of reports and datafiles provides documented, reproducible data analysis and sharing of standardized datasets with the aim follow ILS guidelines.

Our emerging data automation pipeline, from sample-in to report-out is an important element in enabling increasing number, scale and complexity of lipdomics projects and standardization of data.

The LIFS tools and workflows for mass-spectrometry based lipidomics

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T 29

The LIFS tools and workflows for mass-spectrometry based lipidomics Software for lipidomics workflows is still scattered and often not well interconnected. The Lipidomics Informatics for Life Science project[1] bundled the efforts of three groups with both a background in analytical chemistry of lipids and bioinformatics in Germany. Within the project, we developed novel as well as improved existing software to provide solutions for typical lipidomics workflows.

For direct infusion workflows, we improved LipidXplorer's[2] runtime performance, thereby pushing the limit of sample processing into the hundreds of samples and refined existing MFQLs. The novel tool lxPostman allows to perform quantitation on LipidXplorer output in a user-friendly way as an interactive R/Shiny application. The mzTab-M tabular exchange format[3] provides a well-defined metadata, feature, evidence and quantity format which provides the glue between the different steps of the workflows.

The GOSLIN[4] libraries facilitate parsing of existing lipid nomenclature and variants thereof for subsequent normalization and fully automatic mapping to the current shorthand lipid nomenclature as recommended by the Lipidomics Standards Initiative.

For targeted LC-MS workflows, we developed LipidCreator[5], which can be connected to Skyline[6] for targeted lipid assay design and library generation of over 60 lipid classes and up to 10^12 different lipid (sub)species and CE optimization for mediators.

Further downstream, we improved LUX Score[7] to cover a much larger lipid structural space for improved, organism specific, qualitative lipidome comparisons, with new support for lxPostman output as input. For interactive comparison and visualization on both qualitative and quantitative levels, we developed Clover, an interactive R/Shiny application. It supports input from the direct infusion as well as the targeted lipidomics pipeline and performs statistical tests on multi-factor designs on lipid species and subspecies levels.

Most of the tools are already available at lifs-tools.org or from github.com/lifs-tools. They will be made publicly available under open source licenses after publication or on prior request to the authors. Our workflows were already validated and successfully applied in a number of publications.

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2 R. Herzog et al., *PLoS ONE*, Bd. 7, Nr. 1, S. e29851, Jan. 2012, doi: 10.1371/journal.pone.0029851
3 N. Hoffmann et al., *Anal. Chem.*, Bd. 91, Nr. 5, S. 3302–3310, März 2019, doi: 10.1021/acs.analchem.8b04310
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Investigating Global Lipidome Alterations with the Lipid Network Explorer

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T 30

Lipids play an important role in biological systems and have the potential to serve as biomarkers in medical applications. Advances in lipidomics allow identification of hundreds of lipid species from biological samples. However, a systems biological analysis of the lipidome, by incorporating pathway information remains challenging, leaving lipidomics behind, compared to other omics disciplines. An especially uncharted territory is the integration of statistical and network-based approaches for studying global lipidome changes. We developed the Lipid Network Explorer (LINEX), a web-tool addressing this gap by providing a way to visualize and analyze functional lipid metabolic networks. By superimposing statistical metrics onto lipid metabolic networks, it enables a view on global and local changes in the lipidome while simultaneously integrating a data-driven analysis and prior biochemical knowledge.

LINEX utilizes metabolic rules to infer links between lipids. These links can either represent direct enzymatic transformations or heuristics, depending on which type of metabolic rule the respective connection originates from. Rule sets can be defined to resemble various lipid reactions such as head group modifications, fatty acid addition/removal or fatty acid modifications, i.e. elongation, desaturation and hydroxylation. All rules can easily be customized to specific organisms or experimental conditions by users.

LINEX can handle different common levels of fatty acid resolution (sum, molecular, sn-specific) including mixed identification levels within a data set. However, it profits from higher fatty resolution, due to increased accuracy of the matching.

In order to integrate statistical analyses with biochemical knowledge, different metrics for lipids, i.e. p-values and fold-changes, as well as for reactions, i. e. (partial) correlations and changes in correlations between groups, are utilized to visualize quantitative changes and associations.

This way, a global view on lipidomic changes between sample groups is provided.

Using several published data sets, we could demonstrate how LINEX improves the functional analysis and interpretation of lipidomics experiments. Using a study on colorectal cancer [https://doi.org/10.1016/j.bbalip.2019.158579], we were able to link lipidomic changes to potential alterations of MBOAT7 and showed that ether-PE species, which are altered in the tumor, are linked to each other by high correlations, a finding the authors missed. This application case highlights how lipidomics data analysis profits from providing a global metabolic network view and going beyond individual significances.

By providing a novel way of analyzing, visualizing and exchanging lipidomics data, LINEX greatly assists researchers in generating hypotheses and mechanistically understanding the remodelling of lipid metabolism. It lays the foundation for the integration of lipidomics with other omics disciplines and for further improvements in the analysis of lipid metabolic networks going beyond pathway enrichment.

LNEX is available at https://exbio.wzw.tum.de/linex/ as a web-application and at https://gitlab.lrz.de/lipitum-projects/linex as a docker container for local usage. The corresponding research article is published in Metabolites [https://doi.org/10.3390/metabo11080488].

Poster Session

P 01	MALDI-timsTOF MS for lipidomics profiling in infectious diseases	Laura Bindila
P 02	High throughput clinical lipidomics screening and relative quantification of human plasma and serum utilizing MALDI-tims-TOF	Sebastian-Alexander Tölke
P 03	Short-chain fatty acid esters of hydroxy fatty acids – a new gut lipid biomarkers for obesity and influenza infection	Siddaba Savegowda B. Gowda
P 04	The influence of plasma free fatty acids on cognitive function and structural damage of the aging brain	Sebastian Simstich
P 05	Sterolomic profiling of human plasma to reveal altered cholesterol metabolism in Parkinson's Disease	Manuela Pacciarini
P 06	Lipidomics as a tool to highlight the differences in plasma lipid profile of Phenylketonuric children	Inês M. S. Guerra
P 07	Search for novel biomarkers for early breast cancer diagnosis using lipidomics	Kristrun Yr Holm
P 08	Phospholipids as biomarkers for chlorine gas exposure	Petrus Hemström
P 09	Phospholipidomics highlights promising clinical biomarkers in multiple sclerosis	Helena Beatriz Ferreira
P 10	4-D-PASEF quantitative lipidomics for high throughput clinical profiling of human plasma and serum	Raissa Lerner
P 11	Assessment of different Quantification Strategies for Clinical Lipidomic Profiling of Human Plasma	Dhanwin Baker
P 12	New tools for lipidomic profiling in clinical cohorts	Justine Bertrand-Michel
P 13	Benchmarking One-Phase Lipid Extractions for Plasma Lipidomics	Marcus Höring
P 14	Method development for mouse tissues and plasma fluid and lipidomics effects in high-fat diet-induced obese mice using the Lipidyzer platform	Mark Haid
P 15	Application of Lipid Class Ratios for Sample Stability Monitoring – Evaluation of Murine Tissue Homogenates and SDS as a Stabilizer	Sabrina Krautbauer
P 16	Benzoylation of Nonpolar and Polar Lipid Classes in Human Plasma Characterized by RP-UHPLC/MS	Ondrej Peterka
P 17	Bioanalysis of Lipids: Comparison between Direct infusion with Differentialion mobility and reverse-phase ultra-high performance liquid Chromatography-mass spectrometry methods	Z. Zhang
P 18	Determination of polar lipids in cereals by a complementary approach of hydrophilic interaction liquid chromatography (HILIC) and reversed-phase HPLC coupled with high-resolution mass spectrometry	Svenja Schneider
P 19	Lipid quantification by reversed phase liquid chromatography utilizing a counter gradient	Felina Hildebrand
P 20	Non-targeted lipidomics of phospholipids by (UHP)LC-ESI-HRMS – Optimization of chromatographic and QExactive HF-settings	Katharina M. Rund
P 21	Investigations on sphingolipids in Caenorhabditis elegans by two-dimensional multiple heart-cut liquid chromatography – mass spectrometry	Johannes Scholz

P 22	Sphingolipid quantification with species-specific response factors utilizing a KNIME workflow for data processing	Nina Nicole Troppmair
P 23	Determination of Bile Acid Profiles Using OrbiTrap Technology and Comparison with Data Generated by a Liquid Handling System and Triple-Quad Mass Spectrometry	Günter Fauler
P 24 Remote	Sensitive measurement of hydroxy metabolites of Vitamin-D and respective epimers using LC-MS/MS which overcomes challenges of chemiluminescent immunoassay	Suet Ying Lee
P 25	Comparison of ionization techniques for the gas chromatographic analysis of free fatty acids in plasma, serum, and cells	Paul E. Görs
P 26	Determination of double bond positions in methyl ketones by gas chromatography – mass spectrometry using dimethyl disulfide adducts	Matti Froning
P 27	Quantification of free fatty acid derivatives double bond position isomers in plasma samples by shotgun lipidomics	Timo Sachsenheimer
P 28	Studies on pro-resolving lipid mediators upon mPGES-1 inhibition in inflammatory cells	Bing Peng
P 29	Establishment of an UHPLC-MS/MS method for fast and robust quantification of PUFAs and oxylipins in mouse plasma and tissues	Fabien Riols
P 30	Lipotypes of the honeybee Apis mellifera: from in-hive duties to risky tasks	Kathrin M. Engel
P 31	Uncovering the complexity of the yeast lipidome by means of nLC/NSI-MS/MS	Stefanie Rubenzucker
P 32 Remote	Elucidating the effect of diet and probiotic supplementation on the development of atherosclerosis in ApoE knockout mice using LC-HRMS lipidomics	Lise Cougnaud
P 33	Does the phospholipid profile of melanoma-derived exosomes influence cell osteotropic propensity?	Patrizia Lopalco
P 34	Adipose tissue secreted factors induce PPARα signaling in human breast cancer cells altering lipid homeostasis and increasing tumor cell motility via Angptl4	Christina Blücher
P 35 Remote	Eicosanoid measurements in Intercept treated platelet concentrates for evaluation of platelet function in vitro	Gerhard Hagn
P 36	Platelet Lipidomics – Quantitave lipid analysis of human platelets and platelet release	Susanne Heimerl
P 37	Targeting platelet lipids with physical plasmas – unraveling the molecular pattern of plasma-assisted hemostasis	Johanna Striesow
P 38	Lipidome analysis of murine megakaryocytes during maturation	Bianca de Jonckheere
P 39	Quality Control Tools to increase the Confidence in lipid annotations	Sven Wolfgang Meyer
P 40	Goslin 2.0 implements the most recent lipid short-hand nomenclature for MS-derived lipid structures	Dominik Kopczynski
P 41	Lipi Detective: a Deep Learning Model for the Detection of Lipid Species in Mass Spectra	Vivian Julia Würf

MALDI-timsTOF MS for lipidomics profiling in infectious diseases

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P 01

Over the past decade, matrix-assisted laser desorption ionization (MALDI) based time-of-flight (TOF)/mass spectrometry (MS) approaches were successfully developed and optimized to serve as a rapid standard analytical tool for clinical applications, such as pathogen identification based on their protein or peptide compositions.

More recently, the dimension of ion mobility (IM) was coupled to high-resolution mass spectrometry, enabling the separation of isobaric and isomeric analytes by ion-neutral collision cross sections (CCS). Moreover, MALDI coupled to trapped ion mobility time-of-flight (timsTOF) MS improved the sensitivity of MALDI approaches, which were mainly were restricted to the analysis of larger compounds such as proteins. MALDI-timsTOF MS generates dispersions of analyte signals in the mobility space, which allows the detection of low molecular weight metabolites ($m/z < 1500$).

With the MALDI-timsTOF MS application, we aim at lipidomic profiling from small biological sample amounts, such as host- or bacterial cells in infectious disease research.

High throughput clinical lipidomics screening and relative quantification of human plasma and serum utilizing MALDI-tims-TOF

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P 02

Rapid screening of patient samples has become more and more critical in recent years. Quick analyzing of high numbers of patient samples leads to shorter response time and lower cost per sample. Due to the lack of front-end separation of MALDI, discrimination of isobars is rather challenging and limited only to near isobaric peak separation on high mass precision measurements. Ion-mobility tandem MS promises to resolve some drawbacks while combining an additional dimension of separation with the speed of a MALDI and the specificity of an HR-MS.

We developed the following:

[1] Standardized sample preparation and optimized instrument settings for the most common lipid classes have been developed.

[2] Through standards, a spectra library of m/z and CCS has been generated and cross-validated with LC-MS.

[3] Relative quantification via the utilization of internal standards

By applying this strategy in a clinical lipidomic environment, the instrument time can be reduced drastically while also reducing sample uptake.

Short-chain fatty acid esters of hydroxy fatty acids – a new gut lipid biomarkers for obesity and influenza infection

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P 03

Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) are novel endogenous lipids with important physiological functions in mammals, such as anti-inflammatory, antidiabetic, and antioxidant effects. Until now, nearly 50 families of FAHFAs with more than 300 regioisomers have been identified, and the hunting for new species is still going on. We recently uncovered a new type of lipids to this family with the aid of untargeted lipidomics named short-chain fatty acid esters of hydroxy fatty acids (SFAHFAs). Their structures were extensively characterized with MSn analysis using LTQ-Orbitrap-MS, and determination sensitivity was improved with chemical labeling studies. Analysis of colonic contents from high-fat diet-induced rat and influenza infected mouse samples revealed these lipids as novel gut-biomarkers. Especially, a significant rise in their levels almost by 6 folds in influenza-infected samples was observed compared to controls. Due to the lack of authentic standards, studies focusing on SFAHFAs bioactivities were limited. To reveal their biological effects and establish an absolute quantitative method, we constructed a chemical library of SFAHFAs with 55 isomers from C16 to C26 carbon. Also, validated the targeted analytical method for SFAHFAs by establishing single reaction monitoring channels using TSQ-Max. Overall, SFAHFAs analysis techniques, challenges, and future directions necessary to advance this area of research will be discussed.

The influence of plasma free fatty acids on cognitive function and structural damage of the aging brain

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P 04

Background:

It is assumed that cognitive function and structural damage of the aging brain is influenced by the composition of the cerebral ω-3 and ω-6 polyunsaturated fatty acids (PUFAs), but for all that, existing data show inconsistent results.

Methods:

In a retrospective study, we explored the association between free plasma PUFAs concentrations, cognitive function and brain structure atrophy in a well-characterized community-dwelling cohort of elderly individuals without stroke and dementia. Therefore, we analyzed ten different free fatty acids in stored plasma samples from 391 non-demented elderly individuals by gas chromatography mass spectrometry with negative chemical ionization and PFBBr derivatization. Memory, executive function and visuopractical skills were captured with neuropsychiatric tests and were performed in all participants. In a subset of 167 individuals brain atrophy was assessed by MRI.

Results:

Cognitive functions showed no significant association with ω-3 PUFAs concentrations, but lower executive function was significantly associated with higher concentrations of free ω-6 PUFAs ($p=0.042$), and, in particular, linoleic acid ($p=0.01$). Temporal lobe volume was positively related with ω-3 PUFAs. In contrast frontal lobe volume was in inversely related with ω-6 PUFAs. Both associations did not withstand correction for multiple comparisons.

Conclusion:

Our observed associations are weak but they suggest subtle effects of PUFA imbalances on cognition and brain structure. Most sensitive to these imbalances of ω-3 and ω-6 PUFAs are the frontal and temporal lobes.

Structural elucidation of lipid A in Gram-negative bacteria using LC-MS/MS

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P 05

Parkinson's disease (PD) is a neurodegenerative disorder characterised by a progressive and uncontrollable deterioration of dopaminergic cells from the nigrostriatal pathway, resulting in typical motor symptoms like tremor, rigidity and movement imbalance and non-motor manifestations, including neuropsychiatric symptoms. The complex and multifactorial nature of this condition makes the aetiology still uncertain, with no specific and effective treatments available. However, recent findings link disordered brain cholesterol metabolism to PD development, pointing out a plausible central role for sterol molecules in the main PD pathological pathways, represented by oxidative stress, endosomal-lysosomal dysfunction and neuroinflammation.

To shed a light on the contribution of sterols to PD, human plasma samples from PD patients (n=100) and non-PD controls (n=102) have been analysed through a targeted lipidomic strategy focusing on the sterols content.

The method consists of a multi-step procedure starting from sterol extraction, chromatographic separation, enzymatic assisted derivatisation to LC-MS3 qualitative/quantitative analysis.

A total of 22 plasma sterols/oxysterols have been partially or fully identified, ranging a concentration from 0.07 to 700,000 ng/mL in plasma.

Of the 22 plasma sterols/oxysterols 2 cholesterol precursors, 13 hydroxysterols and 6 cholestenoic acids have been identified. Mann-Whitney and Kolmogorov-Smirnov tests were used to compare patients and controls plasma sterols/oxysterol levels.

Identifying relevant differences in the cholesterol metabolite content between PD patients and non-PD individuals could serve as a starting point for novel R&D strategies as well for the identification of clinically significant biomarkers for disease risk, onset, and progression

Lipidomics as a tool to highlight the differences in plasma lipid profile of Phenylketonuric children

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P 06

Phenylketonuria (PKU) is the most prevalent innate error of amino acid metabolism, characterized by elevated levels of phenylalanine (Phe) in the blood, a condition known as hyperphenylalaninemia (HFA). In more than 98 % of the patients, HFA is due to deficiency of the liver enzyme phenylalanine hydroxylase (PAH) that converts Phe into L-tyrosine (Tyr). Accumulation of Phe may cause severe cognitive impairment. Therapeutic approach is very important to prevent irreversible sequelae in phenylketonuric patients and is based on a lifelong low-Phe diet. Dietary restrictions can lead to imbalances in specific nutrients, notably lipids.

Previous plasma/serum and red blood cells studies have reported lipid changes in PKU patients, namely in lipoproteins and fatty acid profile. Despite the evidence that the lipid profile is changed in PKU patients, more studies are needed to understand in detail how lipidome is affected, particularly at the level of phospholipids. Thus, in this poster it will be present the results of the comparison of the plasma phospholipidome of PKU and healthy children (CT) analysed by hydrophilic interaction liquid chromatography-tandem mass spectrometry and gas chromatography-mass spectrometry. Using this approach, 187 lipid species belonging to 9 different phospholipid classes and 3 ceramides were identified. A separation between PKU and CT groups was shown in the principal component analysis of the lipid species data set. Univariate analysis revealed that 146 of 190 lipid species were statistically different between groups. The phosphatidylcholines bearing polyunsaturated fatty acids (PUFA), were the species with major variation and more abundant in the PKU group. The higher level of PUFA-containing lipid species may be related with the PUFA supplementation carried out for some PKU patients.

This study was the first report comparing the plasma phospholipidome of PKU and healthy children, highlighting that the phospholipidome of PKU children is significantly altered when compared with healthy children. However, further studies with larger cohorts are needed to clarify whether these changes are specific to phenylketonuric children.

Search for novel biomarkers for early breast cancer diagnosis using lipidomics

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P 07

Breast cancer (BC) is the most common cancer among women in Western societies and the second leading cause of cancer deaths. Early detection of BC is crucial for increasing survival rates and screening methods play therefore a vital role. Mammography is the most common screening method for early BC detection. Unfortunately, often in the early stages of the BC development the tumor is not visible on these mammographs and therefore BC is often diagnosed at a late stage of tumor development. BC is a heterogeneous disease with diverse patient outcomes dependent on clinico-pathologic variables like tumor size, nodal status, and histologic grade. The most distinct differentiation between BC subtypes relies on hormone receptors status and HER-2 status. Several human BC cell lines share characteristics with human BC tumor subtypes and therefore can be used as an important investigational tool.

The aim of this project is to investigate whether the subtype defined by the transcriptome is reflected in the lipidome of six BC cell lines representing different BC subtypes. An ultra-performance liquid chromatography (UPLC) coupled to a time-of-flight mass spectrometer (QToF) was applied to analyze the lipidome of the BC cell lines. Data analysis was conducted using Progenesis QI software and multivariate data analysis (MVDA) methods were performed using SIMCA software.

Of the six BC cell lines, three represents ER and PgR positive subtype (CAMA-1, MCF-7, T-47D). The SK-BR-3 cancer cell line represents HER2 overexpressing tumors. MDA-MB-231 and MDA-MB-436 are model cell lines for ER, PgR and HER2 negative (triple negative, TNBC) tumors. The MCF10A cells are non-cancerous human mammary epithelial cells and often used as a control cell line for BC. An increased abundance of triacylglycerols (TG) ≥ C-48 with moderate or multiple unsaturation in fatty acyl chains and down-regulated ether-phosphatidylethanolamines (PE) was identified in cell lines representing ER and PgR positive subtype. In the cell line representing HER2-overexpressing subtype, an elevated expression of TG (≤ C-46), phosphatidylcholines (PC) and PE containing short-chained (≤ C-16) saturated or monosaturated fatty acids was observed. Additionally, an increased abundance of PC ≥ C-40 was identified in cell lines of TNBC subtype. These results indicate that subtypes defined by the transcriptome are indeed reflected in differences in the lipidome. In future studies, the similarity of the lipidome of the in vivo culture BC cell lines and actual BC tumor subtypes in clinical plasma samples will be investigated. This could potentially improve early BC diagnosis and patient outcome.

Phospholipids as biomarkers for chlorine gas exposure

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P 08

In the Syrian civil war chemical weapons has been used repeatedly. The vast majority has been chlorine gas attacks mainly employing improvised delivery vehicles such as "barrel bombs" or "elephant rockets" targeting civilians. Forensic methods proving chlorine gas exposure would greatly enhance the probability of bringing the perpetrators of such attacks to justice.

Unsaturated Phospholipids in the pulmonary surfactant are chlorinated by inhaled chlorine gas forming phospholipid chlorohydrins (PL-OHCl). These chlorohydrins could act as biomarkersproving chlorine gas exposure but are difficult both to sample and analyse.

Using MTBE extraction of Broncoalveolar lavage fluid (BALF) and nano LC-HRMS/MS Phosphatidylglycerol chlorohydrins could be detected, up to 72 h post exposure, in chlorine exposed mice. Carry-over and sample stability was however major issues with the procedure.

In a recent publication transesterification with ethanolamine on a solid support (Waters HLB SPE) generate common biomarkers, from all of the diverse PL-HOCl derivatives carrying the same unsaturated fatty acid. Low transesterification yields and blank contamination from the SPE however hampered the analysis and the method showed the same sensitivity and detection time window as analysing intact PL-HOCL.

BALF samples can generally not be taken from victims of a chlorine gas attack since it requires bronchoscopy, initial experiments with Nasal lavage fluid from the upper airways show promising results and blood samples show some elevation but not to a significant degree.

Phospholipidomics highlights promising clinical biomarkers in multiple sclerosis

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P 09

Multiple sclerosis (MSs) is a neurodegenerative and demyelinating disease in which alterations in the lipid metabolism and lipid profile have been associated. Different reports state that the metabolism of arachidonic acid and the profiles of fatty acids, ceramides, phospholipids and lipid peroxidation products are changed in this disease. Yet, the modulation of circulating lipids at the molecular level in MSs remains unclear. In this work, we assessed the serum phospholipidomic variations of patients with MSs. We compared plasma lipidome variation from MSs patients and healthy controls and between disease status (relapse and remission), using an untargeted lipidomic approach (HILIC-high resolution-MS/MS). The results obtained in this study showed variation on some lipid species of PE, PC, LPE, ether-linked PE and ether-linked PC classes in MSs patients when compared with healthy volunteers. PC and PE with esterified polyunsaturated fatty acids, as well as plasmalogen PC and PE species, which are natural endogenous antioxidants, displayed significantly lower relative abundances in patients with MSs (overall disease) and patients in both remission and relapse status of MSs. From all the species identified, only PC(38:1) was statistically different between disease and healthy controls and between disease status, being lower in patients with MSs in remission state. Having said that, PC(38:1) could be a potential biomarker of MSs. The results of this study show for the first time that the serum phospholipidome of MSs significantly differs from that of healthy controls. Additionally, understanding the adaptation of lipid metabolism in this disease may give new perceptions of the disease pathology which could be useful for new therapeutic and diagnostic approaches.

4-D-PASEF quantitative lipidomics for high throughput clinical profiling of human plasma and serum

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P 10

Lipids play essential roles in many physiological processes and pathological conditions, and thus emerge as important candidates for therapeutic agents, biomarker discovery, treatment response, and follow-up monitoring in clinical research. Yet the implementation of lipidomics in clinical research requires still a lot of optimization/adaptation to allow the proper translation from academic laboratories.

Here, we developed a robust 4-D-PASEF analytical workflow:

[1] automated high-throughput sample extraction and processing in 96-well plates using adequate robotic device

[2] UPLC chromatography optimized to achieve efficient LC separation and IMS-MS detection within 20 minutes analysis time.

[3] Annotation strategy based on in-house acquired and generated spectral libraries and scoring system for high-confidence lipid species identification, particularly isomeric and isobaric discrimination. Class-specific standard lipid mixtures containing defined isomeric lipids such as glycerophospholipids were obtained and independently used to optimize annotation accuracy based on the descriptors: retention time (RT), mass accuracy, MS2 fragment spectra, and collision cross-sections (CCs).

Standardized processes were developed and optimized to improve the reproducibility of sample preparation, mass spectrometric data acquisition and multidimensional data analysis. Using such a strategy, plasma and serum lipidome were annotated as well as quantified in order to achieve comprehensive lipidomic pheno-mapping with regard to the clinical practice.

Assessment of different Quantification Strategies for Clinical Lipidomic Profiling of Human Plasma

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P 11

The following research is aimed at developing an advanced lipidomic strategy and workflow for deep sequencing, annotation and quantification of human plasma lipidome using modern high-resolution ion mobility mass spectrometry. These instruments imbibe the modern *PASEF – Parallel Accumulation Serial Fragmentation*technology which aids in the improvement of speed and sensitivity in the analytical measurement along with providing high resolution scan mode.

Here we compare different quantification strategies based on the acquired 4D PASEF data. For this, the analytical method for identification was before-hand validated following the guidelines for the bioanalytical method validation. Limit of Detection (LOD) and Limit of Quantification (LOQ) obtained for major glycerophospholipid categories from the validation experiments were utilized further for optimizing the quantification method. Internally standardised multi Point calibration using external standards was used as the prime quantification method. The NIST Human Plasma reference standard was quantified using this strategy and the results complied with that reported in the literature. The one-point calibration strategy for quantification is also being investigated to eventually replace the multi-point calibration method as it offers high throughput because of shorter sample preparation time and analytical measurement time.

The final quantification strategy will be used to screen the repetitive extracts of NIST reference standard and thus establish the reproducibility and validity of the quantification strategy.

New tools for lipidomic profiling in clinical cohorts

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P 12

Rationale of the work and objectives:

Lipids are essential cellular constituents that have many critical roles in physiological functions. They are involved in energy storage, cell signaling as second messengers, and are major constituents of cell membranes including lipid rafts[1]. Their crucial role is highlighted by their involvement in a large number of heterogeneous diseases such as cancer, diabetes, neurological disorders and inherited metabolic diseases[2,3] it is why it is crucial to be able to profile them easily on large series of clinical samples. Due to the high structural diversity and complexity of lipid species, the presence of isomeric and isobaric lipid species and their occurrence at a large concentration scale, a complete lipidomic profiling of biological matrices remains a challenge specially in a clinical context.

Methods:

First the sample preparation is time consuming and is a source of error so we developed a robotic sample preparation using a TECAN robot (Fluent 780) to improve the robustness and the speed of preparation. And then as it is important to be able to perform a rapid quantitative profiling of the largest panel of families of lipids, we developed and validated an untargeted lipidomic approach by using supercritical fluid chromatography high resolution mass spectrometry on plasma and Dry Blood Spot.

Main results and conclusion:

The liquid-liquid extraction optimized protocol will be presented with its validation on plasma samples Through the building of a homemade lipid data handmade the use of MS-Dial software, we developed an automatic process to produce the relative quantification of around 750 lipids species belonging to the 6 main class of lipids (phospholipides, sphingolipids free fatty acid, lysophospholipid, sterol, acyl carnitine).

These new tools to profiling lipids on plasma or Dry Blood Spot will be presented and discussed. This new service is now available on MetaboHUB-Metatoul facility.

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Benchmarking One-Phase Lipid Extractions for Plasma Lipidomics

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P 13

Clinical lipidomics aims to unravel the role of lipid species in health and disease and to discover novel lipid biomarkers. Essential for successful clinical lipidomics is an accurate and reproducible quantification of lipid molecules. One of the first and crucial steps of a lipidomic workflow is the lipid extraction. Well-established lipid extractions, e. g. Bligh and Dyer[1] or Folch extraction[2], are two-phase extractions based on chloroform. However, numerous studies applied also one-phase extractions to cover a broad range of metabolites.

In this work, quantitative flow injection analysis high-resolution mass spectrometry[3,4] was used to benchmark the lipid recovery of various monophasic extraction methods from human plasma. Organic solvents were selected, to cover a broad polarity range including methanol, ethanol, isopropanol, butanol, butanol/methanol, and acetonitrile. We could observe a loss of nonpolar lipids for polar organic solvents. Furthermore, the ratio of plasma to organic solvent played an important role, and an increase in the solvent volume could enhance the recovery of nonpolar lipid. Thus, the polarity of both the solvent and the lipid class influenced the individual analyte recovery.

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Method development for mouse tissues and plasma fluid and lipidomics effects in high-fat diet-induced obese mice using the Lipidyzer platform

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P 14

The Sciex Lipidyzer™ was developed to quantify up to 1070 lipid species from 13 lipid classes (CE, CER, DAG, DCER, FFA, HCER, LCER, LPC, LPE, PC, PE, SM, TAG) in human plasma samples using a DMS-MS/MS-based stable isotope dilution method. However, biological basic research very often uses murine animal models to study the effects of a gene knock-out/knock-down and specific treatments. Therefore, we have repurposed the Lipidyzer™ assay for lipidomics analyses of murine plasma and tissue samples. After optimizing the extraction protocol, we first validated the adapted assay in terms of precision, accuracy, linear range, LOD, and LOQ for murine plasma, kidney, liver, lung, muscle, brown and white adipose tissue.

In a second step, we evaluated the applicability of the adapted Lipidyzer assay in high-fat diet induced obesity mouse models. Therefore, AdipoQ-Cre-Mgllfl/fl mice with a C57BL/6 background were fed with either chow-diet (CD) or high-fat diet (HFD) for 12 weeks. After dissection, plasma, kidney, liver, lung, muscle, and white adipose tissues samples were extracted with our optimized protocol and measured with the Sciex Lipidyzer™ platform.

The data analysis revealed a tissue specific remodeling of the lipid profiles with a general increase of lipid concentrations in many of the investigated lipid classes.

Interestingly, many concentration changes remained insignificant when viewed from the perspective of the lipid class level (sum of measured lipid species concentrations per lipid class). However, in many instances we found individual lipid species to be significantly changed in either direction even within the same lipid class.

For instance, total TAG levels did not significantly change in any of the investigated matrices upon HFD. However, we observed significant fold changes up to a factor of 16 for those TAG containing an odd number of total carbon atoms across all tissues and plasma. This probably reflects the incorporation of margaric acid (FA 17:0) that was ingredient of the HFD, but not part of the chow diet. Furthermore, the massive increase of specific TAG species in various tissues was mainly limited to TAG with long-chain fatty acids. On the other hand, we observed significantly reduced amounts of TAG containing very long-chain fatty acids in plasma and white adipose tissue.

Our results once again underscore the importance of high-resolution lipidomics to reveal biological alterations in lipid metabolism that might not become apparent on lipid class level.

A Application of Lipid Class Ratios for Sample Stability Monitoring – Evaluation of Murine Tissue Homogenates and SDS as a Stabilizer

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P 15

Introduction:

Lipids are a ubiquitous class of structurally complex molecules involved in various biological processes. In the fast-growing field of lipidomics, preanalytical issues are frequently neglected. Here, we investigated the stability of lipid profiles. Storage of tissue homogenates at room temperature showed substantial alterations of the lipid profiles reflecting lipolytic action. Therefore, ratios of ceramide to sphingomyelin, lysophosphatidylethanolamine to phosphatidylethanolamine, lysophosphatidylcholine to phosphatidylcholine, and diglyceride to triglyceride were applied to monitor sample stability and the effect of sodium dodecyl sulfate (SDS) as a potential stabilizing agent.

Materials and Methods:

Brains, lungs, hearts, spleens, and livers were harvested from C57BL/6 mice (5 female, 6 male), homogenized and sample material was pooled tissue-wise. The effect of sample storage in methanol/water (50/50, v/v) at room temperature and the influence of different SDS concentrations on the stability of lipids was investigated. The analysis of lipids was performed by direct flow injection analysis (FIA), using either a triple quadrupole mass spectrometer (FIA-MS/MS; QQQ triple quadrupole) or a hybrid quadrupole-Orbitrap mass spectrometer (FIA-FTMS; high mass resolution).

Results:

The addition of SDS led to a concentration-dependent stabilization of lipid profiles in liver, brain, and heart homogenates, while in lung and spleen homogenates, in particular, the lysophosphatidylethanolamine to phosphatidylethanolamine ratio increased upon addition of SDS.

Conclusions:

Physical, chemical, and/or enzymatic reactions influence the stability and detection of lipids. Lipid class ratios provide a simple but powerful readout to monitor the stability of samples and evaluate the feasibility of stabilizing agents or conditions. The addition of SDS quenches enzymatic activity and can be used to preserve lipid stability during sample storage and handling. Of note, stabilization is lipid class- and tissue-specific and needs to be evaluated respectively.

Benzoylation of Nonpolar and Polar Lipid Classes in Human Plasma Characterized by RP-UHPLC/MS

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P 16

The character of functional groups in lipids has a significantly effect on the extraction efficiency, chromatographic behavior, and sensitivity, which can be changed by chemical derivatization. Benzoyl chloride is a nonhazardous, commonly available, cheap derivatization agent with a high reactivity for several functional groups. Derivatization reaction, retention and fragmentation behavior were investigated for 4 nonpolar and 8 polar lipid classes. The products were characterized by high-resolution mass spectrometry and MS/MS. The derivatization reaction was thoroughly optimized using spiked pooled plasma, and the molar ratio 4:1 of pyridine with benzoyl chloride reacting for 60 min at ambient temperature provided the best yield. The repeatability and reproducibility were investigated by one operator, resp. two operators, reporting RSD lower than 20 %. The stability of the derivatives was determined at least one month and five freeze/thaw cycles stored at -80 °C by periodical measurements. The comparison of the derivatization and nonderivatization approaches were investigated using calibration curves of 22 internal standards representing 12 lipid classes. The sensitivity expressed by the ratio of calibration slopes was increased by 2 to 10-fold for almost all investigated lipid classes and even more than 100-fold for monoacylglycerols. The limit of detection was decreased 9-fold for monoacylglycerols (MG), 6.5-fold for sphingoid base (SPB), and 3-fold for diacylglycerols (DG). In total, 169 benzoylated lipid species from 11 lipid classes were identified in human plasma using the high confidence level of identification (mass accuracy, MS/MS spectra, and retention time dependences). The derivatization method enables detection of more lipid species for MG, DG, and SPB compared with nonderivatized RP-UHPLC/MS methods.

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Bioanalysis of Lipids: Comparison between Direct infusion with Differentialion mobility and reverse-phase ultra-high performance liquid Chromatography-mass spectrometry methods

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P 17

Introduction:

Lipidomics aims at analysing all lipid species and their biological functions. The structural complexity of lipids and the presence of various isomers require the use of sensitive, high resolution analytical strategies which enable the discrimination between lipid species. Differential ion mobility technology (DMS) allows for the separation of isobaric lipids between different lipid classes and determination of molecular compositions within a single run.

This work compares two lipidomics approaches, direct infusion mass spectrometry (DI-MS) equipped with DMS and reversed-phase ultra-high performance liquid chromatography (UHPLC-MS), both in terms of analytical performance and biological applicability. Both methods were validated for nine lipid classes in plasma samples according to reference guidelines. Furthermore, we compared the biological outcome of both lipidomics platforms.

Methods:

The DI-DMS-MS comprises a Sciex 5500 QTRAP System equipped with DMS and a high flow LC-30AD solvent delivery unit. The UHPLC-MS system used a Waters Acquity UHPLC system coupled to an Agilent QTOF. In house pooled EDTA plasma samples were used for the method validation. Biological study samples came from a study including 25 HIV-positive women.

Preliminary data:

In total, nine lipid classes, i.e., phosphocholines (PCs), phosphoethanolamines (PEs), lysophosphatidylcholines (LPCs), lysophosphoethanolamines (LPEs), sphingomyelins (SMs), cholesteryl esters (CEs), triglycerides (TGs) and free fatty acids (FFAs) were detected using both platforms. 854 and 162 lipid species were reported in the DI-DMS and UHPLC methods respectively. The DI-DMS-MS method provides more comprehensive results due to the determination of fatty chain information, which is not provided by the UHPLC-MS method. The sample consumption of DI-DMS-MS method is 2 times higher than for UHPLC-MS method using 100µl and 50µl starting material, respectively Performance characteristics of each method were calculated using pooled plasma spiked with mixtures of non-endogenous lipids. The

linearity range of standards spiked in the plasma matrix was in most cases two to four orders of magnitude for both methods. Limits of detection were comparable between the two methods and repeatabilities for all species were below 18 % for both methods.

Biological study samples were used to compare individual levels of lipid species measured on the two platforms. Spearman correlation coefficients (r) were calculated to show the correlation between the two platforms. LPC, FFA show good correlations between platforms ($r>0.75$), while CE and LPE showed relatively poor correlations ($0.5<r< 0.65$). These results are supported by the literature. From the biological perspective, human plasma samples from an HIV study were measured using both platforms. Data analysis using linear regression analysis including confounders as covariates was used to find the most relevant metabolites from both platforms. Both platforms provided useful data for group separation, but data from the DI-DMS-MS was able to provide additional information on side chain compositions.

Determination of polar lipids in cereals by a complementary approach of hydrophilic interaction liquid chromatography (HILIC) and reversed-phase HPLC coupled with high-resolution mass spectrometry

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P 18

Lipids have different biochemical functions in plants, which are not yet fully understood. In cereals, lipids are also of interest to consumers due to their contribution to the nutritional value and quality of cereal products. Previous studies have already compared lipid profiles of various cereals in order to find suitable lipid markers for differentiation or in context of authenticity testing of cereals.[1] Due to the methods used, not all lipids were covered or not investigated in detail. The aim of this work is to compare lipid profiles of polar lipid classes that have received less attention so far, such as N-acyl-phosphatidylethanolamines (NAPE) and acyl-monogalactosyldiacylglycerols (acyl-MGDG) in different cereals. While MGDG and digalactosyldiacylglycerols (DGDG) represent a major fraction of membrane lipids in amyloplasts, structural derivatives, such as acyl-MGDG, which are acylated at C-6 position, also appear in cereals. In addition, NAPEs show a relatively high contribution to the total phospholipid content in wheat. The formation of these two interesting lipid classes seems to be dependent on external stress factors in plants.

Given the high complexity of the cereal lipidome, comprehensive characterization of these compounds is a challenging task. Therefore we applied a complementary approach based on two liquid chromatographic separation methods, hydrophilic interaction liquid chromatography (HILIC)[2] and reversed-phase high-performance liquid chromatography (RP-HPLC)[3]. Online coupling with high-resolution mass spectrometry (HRMS) and data-dependent MS/MS experiments was utilized to determine polar lipids in wheat and oat samples.

Due to different separation mechanisms and selectivities of stationary phases, HILIC and RP-HPLC offer complementary strategies for analysis of lipids. While hydrophilic interactions with polar head groups predominate in the HILIC method used, in the RP-HPLC method lipids are separated based on hydrophobic interactions with fatty acid residues. Using this approach, lipid profiles of NAPE, MGDG, DGDG and acyl-MGDG could be determined and compared between these two cereals.

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Lipid quantification by reversed phase liquid chromatography utilizing a counter gradient

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P 19

The use of reversed phase liquid chromatography (RPLC) is challenging in quantitative lipidomics because lipids are separated by their fatty acyl chain on lipid species level. For accurate lipid quantification the co-elution/-ionization of an analyte and internal standard (ISTD) are necessary to ensure the same ionization efficiency. When using RPLC this would require an ISTD for each analyte which is not feasible. To reduce matrix effects due to different elution times of analyte and ISTD we propose to use a counter gradient running parallel to the RP gradient to keep the solvent composition during ionization constant for all lipids over the whole gradient.

With flow injection analysis it was shown that the solvent composition has an effect on the ionization efficiency of lipids. This matrix effect was eliminated by introducing a counter gradient which leads to an ionization efficiency independent of the solvent composition during ionization.

The counter gradient was introduced to a RPLC separation of lipids which was downscaled to a microflow setup to ensure high sensitivity despite dilution effects due to combining two LC flows. The quantification accuracy of the method was assessed by quantifying a lipid standard mix and a lipid extract of SRM 1950 (NIST, USA) with a small number of deuterated lipid class specific ISTDs (one ISTD per lipid class). Quantitative values were compared to the known concentration of lipid standards and consensus values of SRM 1950.

In conclusion, introducing a counter gradient into a lipid RPLC separation eliminates the matrix effect of different solvent compositions during ionization and can lead to an improved quantification accuracy in RPLC-MS lipidomics.

Non-targeted lipidomics of phospholipids by (UHP)LC-ESI-HRMS – Optimization of chromatographic and QExactive HF-settings

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P 20

Lipids are essential for life exhibiting various biological functions in the organism. In addition to their importance in energy storage, they are central components of cell membranes being crucial for their structure and the transmission of signals. In particular, polar lipids such as phospholipids and sphingolipids are involved in multiple signal transduction processes. The composition of lipids is highly dynamic and affected by lifestyle and diet as well as pathophysiological processes such as inflammation. Owing to their role in health and disease the comprehensive analysis of lipids particularly by using non-targeted LC-MS based techniques is a promising tool in biomarker research. To obtain meaningful and reliable results extensive method development and characterization of its performance is required.

Here, we thoroughly optimized a non-targeted LC-ESI-HRMS (QExactive HF) method using acquisition in full MS data-dependent MS2 (FullMS/ddMS2) mode with a focus on phospholipids. Method characterization included optimization of source parameters such as voltages and gases to improve signal intensity and spray stability. Chromatographic separation was optimized regarding the selection of the C18 column (2.1x100 mm, 1.7 µm) and gradient to ensure elution of the phospholipids over a preferably broad retention time window. Stepped TOP N experiments of the FullMS/ddMS2 method over the chromatogram based on peak widths allow fragmentation of as many different precursors to achieve comprehensive tentative identification while ensuring enough data points for semi-quantitative evaluation using normalization to SPLASH internal standard mixture. Lipid extraction from plasma by liquid-liquid extraction with MeOH and MTBE yielded appropriate inter- and interday extraction efficiencies for the lipid classes covered by the SPLASH internal standard mixture as well as reproducible semi-quantitative results for selected phospholipids.

Finally, the method was applied to characterize the phospholipids species which are strongest modulated in response to n3-PUFA supplementation in human plasma.

Sphingolipid quantification with species-specific response factors utilizing a KNIME workflow for data processing

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P 21

Sphingolipids are composed of a hydrophobic moiety, consisting of a sphingoid base (also termed long chain base; LCB), and an alkyl chain (also termed fatty acid; FA), as well as a hydrophilic portion. In the simplest case the hydrophilic part is a hydroxyl group, giving ceramides. Sphingolipids are a diverse category of lipids and play numerous biological roles, acting not only as structural components, but also are key players signaling pathways[1]. Understanding the contribution of sphingolipids to disease processes, necessitates, in addition to their identification, also their quantification.

We used multiple ceramide standards, differing in LCB and FA, to determine the influence of these structural differences on their ionization efficiency and fragmentation. As previously observed, applying the same collision energy to ceramides, with varying chain lengths of the FA, results in altered signal response[2,3]. Across the range of commonly occurring chain lengths, these differences are minor and may, thus, be compensated for by optimizing the collision energy for the specific species, as described before[3]. In contrast, structural differences of the LCB, such as saturated and unsaturated species, led to a significantly different fragmentation pattern. Therefore, ideally internal standards with different LCBs should be included for accurate quantitation, which, however, is not feasible due to the high diversity of sphingolipids. Using one internal standard per lipid class, we utilize responds factors obtained from calibration curves, to consider these differences in signal intensity.

To automatize data processing after acquisition, such as taking responds factors into account, a workflow was developed using the software tool "Konstanz Information Miner"[4]. Overall, the herein described optimization and KNIME workflow supports the comprehensive liquid chromatography-mass spectrometry-based analysis of sphingolipids in biological samples, calculating the lipid concentration in the samples and visualizing the identified lipids based on their building blocks. The workflow has proven suitable for processing data from targeted sphingolipid analyses of complex samples, such as fat cells[5], giving the same quantitation results, as manually obtained from the same data set, in 1/100 of the time.

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Investigations on sphingolipids in Caenorhabditis elegans by two-dimensional multiple heart-cut liquid chromatography – mass spectrometry

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P 22

Sphingolipids gained increasing attention concerning their involvement in bioactive cell signalling and their association with neurodegenerative and cardiovascular diseases. In this poster, we present a novel two-dimensional multiple heart-cut liquid chromatography method (2D-LC) with subsequent high resolution-mass spectrometric detection for the analysis of the three prominent sphingolipid classes: Ceramides, hexosylceramides and sphingomyelins. Often the sample preparation for sphingolipid analysis involves a laborious and unspecific alkaline depletion step of the glycerophospholipids, which can be eliminated with the here presented 2D-LC method. The nematode *Caenorhabditis elegans (C.elegans)* is often used as a model organism in biological studies or studies concerning neurodegenerative diseases and drug discovery. A lipid extract of the C. elegans sample was separated in the first dimension (^{1}D) utilising hydrophilic interaction liquid chromatography (HILIC). This separates the lipids according to their polar headgroups and thus according to the lipid class. After the separation in the 1D-HILIC, the sphingolipid fractions were cut out and stored in a sample loop. Afterwards, a separation based on the chain length was carried out using reversed phase liquid chromatography as the second dimension (^{2}D). An identification via retention time, accurate mass and tandem mass spectrometry was conducted to identify a total of 45 sphingolipids in the C. elegans lipid extract.

Determination of Bile Acid Profiles Using OrbiTrap Technology and Comparison with Data Generated by a Liquid Handling System and Triple-Quad Mass Spectrometry

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P 23

Background:

Bile acids are products of cholesterol catabolism with one to three (all α-) hydroxy- groups. The role of bile acids in cell metabolism, membrane biology and cell signalling is increasingly recognized and their importance in an ascending number of different diseases in many organs, culminating in a so called "gut-host axis", is under rising investigation.

Two primary bile acids, cholic acid (CA) and chenodeoxycholic acid (CDCA), are synthesized in hepatocytes from cholesterol and further conjugated with glycine or taurine.

These bile acids play an important role in digestion and absorption of dietary lipids within the intestine as well as in their, bacterial driven, deconjugation and dehydroxylation into the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA). Further partial reabsorption of all bile acids leads to their entry into the enterohepatic circulation. To a small extend CDCA can be epimerized to 3α-, 7β- ursodexycholic acid (UDCA), normally used as a drug in cholestatic liver diseases such as primary biliary cirrhosis, or in patients with chronic heart failure.

Increasing interest in specific bile acid species makes it necessary to develop robust and comprehensive methods to measure the whole profile of common and rare bile acids. Automated liquid handling systems eventually can help in sample preparation to minimize personal resources.

Methods:

Deuterated internal standards (2 nmol each) and acetonitrile were added to 10 µL of plasma or serum, (or 1 ml of urine, or 10 mg of dried stool, after extended sample preparation). After vortexing and centrifugation of plasma proteins, the supernatant was brought to dryness; samples were redissolved in mobile phase and injected into hplc. Chromatographic solution of isobaric components was performed using a C18-column with an acetonitrile/water gradient and formic acid and ammonium acetate as modulators.

All bile acid subgroups were detected by full-scan analysis in high resolution on an OrbiTrap instrument (Q-Exactive, Thermo Scientific), as well as by three different MRM-experiments on a QTrap 4500MD mass spectrometer (Sciex).

Results:

Calibration graphs for all components were established from 0.05 up to 100 µmol/L and all methods were validated according to common guidelines.

The data from the different mass selective methods are completely comparable, and also automated liquid handling systems can assist in sample preparation.

Sensitive measurement of hydroxy metabolites of Vitamin-D and respective epimers using LC-MS/MS which overcomes challenges of chemiluminescent immunoassay

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P 24

Vitamin-D is a group of lipophilic secosteroids that have been associated with several pathologies. Vitamin-D status is assessed by measuring two main circulating metabolites: 25-hydroxyvitamin-D3 [25(OH)D3] and 25-hydroxyvitamin-D2 [25(OH)D2]. Conventionally, chemiluminescent immunoassays have been used for vitamin-D analysis. However, LC-MS/MS allows specific quantification of both metabolites, which is of importance for adequate stratification. Epimers differ only by the spatial arrangement of the C3-hydroxyl, making chromatographic separation challenging. This study described an LC-MS/MS method for baseline separation and sensitive measurement of four 25(OH)D metabolites in human serum.

Comparison of ionization techniques for the gas chromatographic analysis of free fatty acids in plasma, serum, and cells

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P 25

Fatty acids are essential for biological systems because of their role in energy storage and metabolism, as well as a building block of complex lipids. Moreover, free fatty acids (FFA) can act as signal molecules. Because of their importance, a qualitative and quantitative analysis is critical for understanding biological systems. However, FFA in high concentrations can also be toxic to cells and therefore, FFA are often present in significantly lower concentrations. Considering the low concentration in the nanomolar to the micromolar range together with the complex matrix, the analysis of free fatty acids is still a challenge and requires high sensitivity, a wide linear range, and the ability to detect a large number of saturated as well as unsaturated fatty acids.

Usually, fatty acids are analyzed by gas chromatography after derivatization to the corresponding methyl esters using GC coupled with flame ionization detector (FID) or electron impact ionization mass spectrometry (EI-MS). However, for the selective analysis of FFA, a derivatization using pentaflourobenzyl bromide (PFB) offers an alternative method with less sample preparation. In addition, derivatization allows ionization of FFA in negative mode, resulting in lower background noise and thus in an improvement in selectivity and sensitivity compared to other ionization techniques. Here we compared three different GC-MS methods that were using either atmospheric pressure chemical ionization (APCI), atmospheric pressure photoionization ionization (APPI), and negative chemical ionization (NCI) for the analysis of FFA after derivatization to the PFB esters in negative ionization mode.

All methods used the same GC parameters and covered a wide range of 75 different fatty acids from FA 6:0 to FA 26:6 including all important unsaturated FFA. For comparison, all methods were characterized according to European Medicines Agency guidelines for bioanalytical method validation and figures of merit were determined (e.g. LOD, LOQ). For APCI and NCI the method showed promising results while APPI showed low sensitivity. The LOD for APCI and NCI were between 30 and 100 nM (~3 10 fmol on column) for most fatty acids. Subsequently, the methods were applied to analyze FFA in human plasma, human serum, and cell samples. The samples were extracted according to Matyash *et al.* [J Lipid Res.; 2008; 1137-46] for quantification of FFA, and the lipid extract obtained was subsequently derivatized with PFB. Overall, both methods using APCI and NCI showed similar results with regard to the detected FFA and the

quantified amount. The most concentrated FFA were FA 16:0, FA 16:1, FA 18:0, FA 18:1, FA 18:2, FA 18:3, and FA 20:4 with concentrations ranging from 10 to 140 µM. A comparison with literature data [NISTIR 8185] shows that the same FFA were found in comparable concentrations in previous studies, demonstrating the performance of the method.

Determination of double bond positions in methyl ketones by gas chromatography – mass spectrometry using dimethyl disulfide adducts

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P 26

Methyl ketones are used in the fragrance, flavour, pharmacological and agrochemical industries. The industrial production of these compounds is based on hydrocarbons derived from petroleum. To achieve a sustainable production of methyl ketones, intense research has been carried out to rearrange the fatty acid metabolism of different microbes[1]. In this regard, fatty acid-derived methyl ketones are of interest for the application as biofuels. The biofuel properties and possible further chemical modifications of these methyl ketones are influenced by their chain length as well as their degree of unsaturation and the corresponding double bond position.

Since the overall necessity of the structural elucidation of lipids down to the double bond position is rapidly growing, there is an increasing number of mass spectrometric approaches for this task. Methods are based on different procedures, e. g. ozone induced fragmentation, the Paternò-Büchi reaction, oxidation through osmium tetroxide or dimethyl disulfide adducts. For volatile substances like methyl ketones, methods centered on gas chromatography – mass spectrometry are predestined. Therefore, we chose an approach that utilizes the alkylthiolation of the double bond using dimethyl disulfide and an iodine catalyst. The mass spectrum of the bis(methylthio)-derivatives shows intense ions due to the cleavage at the activated bond of the two carbon atoms of the original double bond[2].

In this work, we localized the double bond position of different medium chain length methyl ketones originating from the gram-negative bacterium *Pseudomonas taiwanensis* VLB120. We demonstrated that dimethyl disulfide derivatives of methyl ketones yield isobaric fragment ions for different double bond positions that need high-resolution mass spectrometry to be distinguished. For example, the dimethyl disulfide adduct of pentadecenone shows similar fragment ions for the ω 9 position and the ω 7 position. We determined the position of all methyl ketones deriving from *Pseudomonas taiwanensis* VLB120 to be the same counting from the end of the aliphatic chain and being ω 7.

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Quantification of free fatty acid derivatives double bond position isomers in plasma samples by shotgun lipidomics

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P 27

Structural elucidation of unsaturated fatty acids using charge-remote derivatization was reported to allow for a highly sensitive identification, quantification and characterization including the determination of double bond positions via characteristic reporter fragments (Bollinger et al. 2010 and 2013, Yang et al. 2013) following charge-switch derivatization using N-(4-aminomethylphenyl)pyridinium (AMPP). Here, we show a workflow to apply this charge-switch derivatization for the analysis of plasma-derived free fatty acids. An iso-octane extraction was performed in the presence of fatty acid standards, followed by the AMPP-derivatization. AMPP-derivatized fatty acids were measured using a QExactive in FullMS mode and via PRM inclusion list MS^2. The acquired spectra were evaluated using LipidXplorer (Herzog et al. 2013) with designated mfql files to also cover the proximal reporter fragment specifiying the double bond position with unsatured fatty acids.

In conclusion, this workflow enables to determine and quantify fatty acids including double bond positional isomers in a well-established and rapid direct infusion-based experimental setup.

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Studies on pro-resolving lipid mediators upon mPGES-1 inhibition in inflammatory cells

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P 28

Microsomal Prostaglandin E Synthase 1 (mPGES-1) is the key enzyme for the generation of the pro-inflammatory mediator prostaglandin E_2which contributes to several pathological features of rheumatoid arthritis (RA). Inhibition of mPGES-1 has been demonstrated to provide efficient anti-inflammatory effects in preclinical models of RA. Importantly, the activation of resolution has been suggested as an alternative way to treat and cure chronic diseases including RA. In contrast to the suppressive effects of NSAIDs, the upstream cyclooxygenase (COX) inhibitors, our research has recently suggested that mPGES-1 genetic deletion or pharmacological inhibition activate cardioprotective and pro-resolving pathways.

In this project, we studied 1) the biosynthesis of pro-resolving cyclopentenones (15d-PGJ2 and its derivatives) and their actions following mPGES-1 inhibition in inflammatory cells relevant to RA and 2) the effects of mPGES-1 vs COX inhibition on arachidonic acid cascade in different model systems: RAW 264.7 and A549 cell lines, mouse primary bone-marrow-derived macrophages and RA synovial fibroblasts.

We observed 1) that the biosynthesis of cyclopentenones in RAW 264.7 was enhanced through the treatment with mPGES-1 inhibitor 118. 2) The mPGES-1 inhibitor 118 downregulated mPGES-1 activity and suppressed the production of PGE_2. 3) In A549, we found that PGD_2 and PGF_{2alpha} were increased after stimulated with IL-1beta and with 118, in comparison to IL-1beta stimulation or IL-1beta plus COX-2 inhibition. RASF were induced by IL-1beta and TNF_{alpha} and further treated with 118, the inhibition of mPGES-1 altered prostaglandin profiles by increasing the production of 6-keto-PGF_{1alpha} (the stable hydration product of prostacyclin) and PGF_{2alpha}. BMDM were treated with LPS and 118, resulting in the increasement of 15d-PGJ_2, PGD_2, TXB_2 and PGF_{2alpha}. 4) The treatment with the COX-2 inhibitor NS-398 suppressed the production of all prostaglandins. The data suggested that the treatment with mPGES-1 inhibitor 118 suppresses pro-inflammatory PGE_2 while maintains the anti-inflammatory and pro-resolving prostaglandins.

Establishment of an UHPLC-MS/MS method for fast and robust quantification of PUFAs and oxylipins in mouse plasma and tissues

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P 29

ω 3/ω 6-PUFAs and their pro- and anti-inflammatory derivatives eicosanoids or docosanoids are important signaling molecules involved in the coordination of many physiological and pathological processes. For research purposes we established a fast and robust UHPLC-MS/MS method that is capable of quantifying 57 oxylipins and PUFAs in numerous mouse matrices within 8 min acquisition time. The extraction protocol was optimized for mouse plasma as well as for various tissues including liver, skeletal muscle, lung, heart, spleen, kidney, brain, brown (BAT) and white adipose tissue (WAT). Extraction recoveries varied between 66 % and 110 % depending on the standards.

We then applied this method to study the effects of an endotoxic shock in lipopolysaccharide-treated (LPS) C57BL/6J mice in comparison to a PBS control (n=6, per group). Mice were sacrificed 18h after LPS/PBS injection. Results showed tissue specific profiles, notably with compounds detected only in one tissue such as 11 β-PGE and 6(S)-LXA_4 in BAT, 8-iso $PGF_{2\omega}$ in heart and LTD4 in lung. As previously published, the most abundant amounts of PGD2 were found in the brain.

Moreover, our results also revealed treatment specific profiles. For instance, 18h after LPS injection, PGE2, a pro-inflammatory prostanoid also involved in the initiation of resolution, was increased in all tissues, especially in eWAT and brain samples. On the other hand, leukotrienes (pro-inflammatory compounds) were lower in LPS lung and plasma samples, but higher in LPS heart samples. Lipoxins, 6(R)- and 6(S)-LXA4, involved in the resolution of inflammation, were increased in LPS adipose tissues.

Our results show that the established UHPLC-MS/MS method can be used to analyze and quantify pro- and anti-inflammatory mediators in numerous mouse tissues and plasma for research purposes.

Lipotypes of the honeybee Apis mellifera: from in-hive duties to risky tasks

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P 30

Honeybees that belong to the same colony display a near-homogeneous genomic background together with a high level of behavioral and physiological plasticity and, thus, are an ideal model for functional lipidomics studies. Due to their nutritional specialization, the only external source of lipids is pollen. However, the diet of bee queens differs from that of workers. Queens are fed glandular secretions provided by the workers. The lipids of theses secretions mainly contain saturated and monounsaturated fatty acids (FA). The comparison of the FA composition of queen and worker bee tissues revealed that FA of queens are mainly monounsaturated, whereas unsaturation increases in workers with pollen consumption. The high amount of saturated FA could in part explain the extended longevity of honeybee queens, in accordance with the "oxidative stress" or "free radical"-theory of ageing.

In the worker caste, there are different adaptations to physiological specializations, which follow a loose chronological sequence ("chronological polyethism"). Newly-emerged worker bees usually start as cell-cleaners, before feeding brood. Leaving the hive for foraging is typically the last step of temporal polyethism. At the transition from in-hive duties to the risky task of foraging, worker bees strongly decrease in weight and storage proteins, whereas the masses of their flight muscles increase. There are also subgroups of similar age but differing specializations, such as „cell heaters" using the flight muscles to produce heat and non-heaters. The flight muscle of honeybees has one of the highest maximum rates of energy turnover of any animal tissue studied so far. Therefore, cell heaters offer an interesting model for functional lipidomic research, because they can be expected to have a high energy turnover and can be compared to same-aged, non-heating individuals. Thus, the present study aimed to elucidate the lipid composition of flight muscles from differentially specialized bees. Our results show that differences in the lipid class of cardiolipins reflect the difference in energy turnover between heating and non-heating hive bees. A near-comprehensive set of A. *mellifera* lipotypes will be presented.

Uncovering the complexity of the yeast lipidome by means of nLC/NSI-MS/MS

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P 31

Nano liquid-chromatography/nano-electrospray ionization mass spectrometry (nLC/NSI-MS) is routinely applied in proteomics research due to its increased measurement sensitivity and protein coverage[1]. However, nLC/NSI-based mass spectrometry methods have hardly been used in the lipidomics community, although their favorable characteristics have also been demonstrated for lipid analysis[2].

Here, we use a nLC/NSI-MS/MS method combined with a semi-autonomous data analysis workflow to probe the lipid landscape of *S. cerevisiae*, a eukaryotic model organism widely used for the investigation of fundamental cellular processes. Using this approach, we were able to identify almost 900 lipid species across 26 lipid classes, including glycerolipids, glycerophospholipids, sphingolipids and sterol lipids. Similar to previously published results[3,4,5], we observed that most lipid classes are dominated by only a few highly abundant species. However, due to the high sensitivity of nLC/NSI-MS/MS, we were also able to demonstrate that a multitude of lower abundant lipids contribute to the complexity of the yeast lipidome. Furthermore, we found odd-chain and diunsaturated fatty acyl moieties to be commonly incorporated in multiple lipid classes, which was not yet reported in previous publications. Lastly, our exhaustive data analysis workflow revealed the presence of putative novel lipid species such as MMPs (mono-methylated phosphatidylserine), not yet described in yeast. Overall, we were able to increase the number of lipid identifications 4-fold compared to previous approaches[3,4,5], highlighting the use of nLC/NSI-MS/MS methods for in-depth investigations of lipidomes.

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Elucidating the effect of diet and probiotic supplementation on the development of atherosclerosis in ApoE knockout mice using LC-HRMS lipidomics

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P 32

Low carbohydrate – high protein (LCHP) diet is used to reduce obesity, an important risk factor for cardiovascular diseases. However, this diet promotes the formation of atherosclerotic plaques in animal model to an even greater extent than high-fat Western-type diet (WD). The cholesterol involvement in atherosclerosis induced by the LCHP diet is refuted by several studies. As a result, the underlying mechanism for higher atherogenicity of LCHP diet is not currently known. Studies using anti-inflammatory probiotics from *Bifidobacterium* and *Lactobacillus* genera demonstrated strong anti-atherosclerotic properties including anti-inflammatory or hypolipemiant activity. The objective of the current study was to examine the possible role of oxylipin inflammatory pathways in the atherosclerosis induced by the LCHP diet and to characterize lipid dysregulation induced by the LCHP diet in ApoE knockout mice model. Male mice (n=12) were fed with the LCHP, WD or control diets for 6 weeks with or without anti-inflammatory probiotic supplementation (*Lactobacillus helveticus*, *Bifidobacterium biffidum*) at two different doses: 0.5 B/day and 5 B/day. Plasma samples (15 µL) collected at 1 and 3 weeks were analyzed by targeted oxylipin method using C18 solid-phase extraction followed by ultra-high performance liquid chromatography separation with an Agilent Eclipse plus C18, (1.8 µm, 2.1 mm x 100 mm) column and quadrupole-time-of-flight HRMS. The method was successfully modified to ensure its compatibility with limited sample volume of 15 µL, while maintaining good oxylipin coverage and similar method precision range (4-12 %, n=6). Plasma samples collected at 6 weeks were analyzed by our untargeted lipidomics method using a liquid chromatography with XSelect CSH C18 column (130 Å, 2.5 µm, 2.1x75 mm) coupled to a LTQ-Orbitrap Velos mass spectrometer in positive and negative electrospray (ESI) with data-dependent MS/MS acquisition. Lipid identification was performed using LipidSearch 4.2 software. Oxylipin results to date shows that oxylipins derived from linoleic acid were significantly affected by the diet and the probiotic administration even at early timepoints. PGF2α was also elevated in the LCHP diet even at 1-week timepoint. This oxylipin is linked to high blood pressure and promotes the formation of atherosclerotic plaque. The combination of the targeted oxylipin and untargeted lipidomics methods provides a global picture of the alteration of lipids during atherosclerotic plaque development induced by the LCHP diet, compares it to control and Western diets and examines how these pathways were modulated by the selected probiotics.

Does the phospholipid profile of melanoma-derived exosomes influence cell osteotropic propensity?

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P 33

Over the last twenty years, the role of exosomes (EXOs) as mediators of intracellular and intercellular communication has emerged. EXOs appear to be involved in various diseases including infectious diseases, neurodegenerative disorders and cancer. Among various causes that can promote cancer progression, there is the alteration in lipid metabolic enzymes and their pathways. EXOs carry bioactive lipids, which trigger cell-to-cell signaling, but the lipid-related aspects of EXOs have not obtained sufficient attention. In the present study, we compare the lipid profiles of two different melanoma cell lines (LCP and SK-Mel28) and we also examine the lipid composition of EXOs derived from the respective cell lines; MALDI-TOF/MS lipid analyses have been performed on very small amounts of intact parent cells and EXOs, by skipping lipid extraction and separation steps. The phospholipid composition of EXOs was found to be significantly different from that of parental cells. Sphingomyelin (SM), lysophosphatidylcholine (LPC) and phosphatidic acid (PA) are enriched lipid species in the membrane of EXOs, underlining the presence of raft-like domains necessary for cell-cell communication and cell signaling functions; in addition, we could unambiguously detect bis monoacylphosphatidic acid (BMP), a specific lipid marker of intracellular membranes (endosomes), as abundant component in the membrane of EXOs. The basic knowledge of lipid species (quality and relative abundance) present in the cell membranes (and in specific intracellular compartments) of cancer cells may help in developing new drugs and pharmacological approaches to affect cell survival and cell death pathways. The possible role of EXOs as carriers of bioactive lipids, such as polinsaturated fatty acids and lysophospholipids, is discussed.

Adipose tissue secreted factors induce PPARα signaling in human breast cancer cells altering lipid homeostasis and increasing tumor cell motility via Angptl4

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P 34

Obesity and high levels of dietary fat intake have been associated with a higher risk of breast cancer development and progression. Recent studies indicate that adipose tissue influences breast cancer cells and promotes cancer progression by secreting chemokines, growth factors and fatty acids. However, the detailed molecular mechanisms by which breast cancer cells and fat tissue affect each other, remain elusive. Here, we investigate the interaction between human breast tumor cells and adipose tissue in a 2D co-culture system and by culturing breast cancer cells with adipose tissue-conditioned media (ACM). Genome-wide gene expression analysis revealed that co-culture with adipose tissue from high-fat diet induced obese C57BL/6 mice up-regulates genes involved in inflammation and lipid metabolism in triple-negative (TN) breast cancer cells, with many of them being known targets of PPAR nuclear receptors, known master regulators of cellular lipid homeostasis. Interestingly, similar results were obtained by treating breast cancer cells with ACM from overweight or obese female patients. Fourier transform mass spectrometry revealed that free fatty acids were most abundant in ACM, especially in ACM prepared from obese adipose tissue. Furthermore, GC-MS analysis showed that oleic, linoleic and palmitic acid were most abundant in ACM, with significantly higher concentrations in ACM stemming from obese patients. These fatty acids are known ligands for PPAR nuclear receptors. Additionally, the major energy producing pathways, β-oxidation and glycolysis were significantly increased in TN cells upon cultivation with ACM. Furthermore, ACM treated breast cancer cells pronounced a more aggressive phenotype, including increased wound healing and invasion capabilities. The ACM induced cell invasion was mediated by Angiopoietin-like 4. Together, our data indicate that interaction with adipose tissue leads to changes in PPAR regulated gene expression and lipid homeostasis in TN breast cancer cells and induces a more aggressive breast cancer cell phenotype.

Eicosanoid measurements in Intercept treated platelet concentrates for evaluation of platelet function in vitro

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P 35

Introduction:

To prevent pathogen transmission *via* blood transfusion, blood products such as platelet concentrates (PCs) are receiving pathogen inactivation (PI) treatments. Platelets may produce eicosanoids, known to be biologically active at very low concentration. In this project, we investigated whether eicosanoid formation differed with regard to two different established PI techniques, which could be related to different tolerability in patients receiving PCs. Therefore, PCs of 15 healthy donors were collected and divided into two single units. While one unit was Intercept treated using amotosalen/UVA, the other unit was receiving gamma irradiation. Both units were stored for seven days at room temperature under constant agitation. Samples were collected at day 1 (untreated) and at day 2, 5 and 7 of each unit.

Methods:

Eicosanoids were enriched by solid phase extraction prior to LC-MS/MS analysis. Separation and detection of analytes was achieved with an UHPLC system (Vanquish™, Thermo Scientific™) equipped with a reversed phase column coupled to a high-resolution mass spectrometer (QExactive™ HF, Thermo Scientific™). The TraceFinder software package (Thermo Scientific™) was used for relative quantification.

Results and Discussion:

As a result, the PI treatments of PCs resulted in significant differences in their eicosanoid patterns. For example, the extracted ion chromatogram (EIC) of m/z 303.2330 representing arachidonic acid (AA) is showing a newly formed isomer at 12.75 min only detected in the Intercept unit, representing an immediate effect of the UVA treatment. The isobars are representing trans-AA isomers, which were successfully synthesized *in-vitro via* UVA illumination of AA in isopropanol including β-mercaptoethanol (according to[1]). The Intercept treatment also induced the formation of hydroxyeicosatetraenoic acids (HETEs), most plausibly via oxygen radicals.

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Platelet Lipidomics – Quantitave lipid analysis of human platelets and platelet release

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P 36

Previous as well as emerging data emphasize the significant role of lipids in platelet physiology. While lipid compositions of resting and activated platelets as well as specific release of specific lipids have been published, a comprehensive set of human quantitative of bulk lipid classes of resting and activated platelets and their lipid release is not available so far.

Therefore, we isolated platelets from 12 (6 male, 6 female) healthy human donors and performed quantitative lipidomics on resting and thrombin-activated platelets as well as on the platelet lipid release upon thrombin activation. Lipid analysis was performed by direct flow injection analysis (FIA), using a triple quadrupole mass spectrometer (FIA-MS/MS)[1,2] and a hybrid quadrupole-Orbitrap high resolution mass spectrometer (FIA-FTMS)[3,4].

In basal platelets, we found, in addition to a high amount of free cholesterol (FC), phospholipids as the most abundant lipid class. To elucidate lipid alteration upon platelet activation, we additionally analyzed lipid class and lipid species profiles in platelet lipid release after thrombin stimulation and platelets after activation. In addition to cholesteryl ester (CE), FC and trigylcerides (TG), which are most probably plasma components released from the open canalicular system (OCS) upon shape change, we found predominantly phospholipids ejected by activated platelets, which were specific for certain phospholipid species.

The validity of the data was checked by comparing the amount of lipids in basal platelets with the total amount of lipids released and left. Here we could confirm as expected a degradation of specific phosphatidylcholine (PC) and phosphatidylinositol (PI) species, in particular PC 36:4, PC 38:4 and PI 38:4. This fits very well to the proposed role of these species as sources of arachidonic acid and their hydrolysis to receptive lysolipids, which could be evidenced by increased concentration of lysophosphatidylcholine LPC 16:0 and LPC 18:0.

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Targeting platelet lipids with physical plasmas – unraveling the molecular pattern of plasma-assisted hemostasis

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P 37

Upon vessel wall injuries, the physiological response process, called hemostasis, results in its final stage in thrombus formation at incision site[1]. In some surgical procedures, blood clotting is artificially induced by electrical currents or heat. Although very effective, reported adverse effects include rejection of blood clots and risk of rebleeding, especially in anticoagulated patients[2]. In such cases, alternatives are in high demand.

Physical plasma is a rich source of reactive oxygen and nitrogen species accompanied by electric fields and ultraviolet light. Among these hydrogen peroxide, nitric oxide and short-lived species like hydroxyl radicals, atomic and singlet oxygen are of the highest relevance. Plasma-derived reactive species interfere with signaling cascades by interacting with proteins, peptides and lipids, triggering – direct or indirect – receptor activation and downstream signaling cascades[3].

It has been observed that physical plasma stimulates blood coagulation with platelets playing a central role[4]. In this study, the impact of a plasma treatment on the platelet lipidome was investigated. A purified platelet suspension was treated with an argon plasma jet and dose and species dependent platelet activation was observed via CD62P expression. Lipids were extracted by a modified MTBE method and analysed by high resolution LC-MS[2]. Our results show, that 12 lipids of the donor-specific lipidome are significantly up- or downregulated after plasma treatment. We found that diacylglycerols, ceramides and phosphatidylethanolamines are among the most influenced lipid classes. Additionally, some oxylipins (thromboxanes) concurrently with lipid oxidation products were elevated.

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Lipidome analysis of murine megakaryocytes during maturation

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P 38

Megakaryocytes (MKs) are large polyploid and highly specialized precursor cells localized within the bone marrow, that function to produce and release blood platelets into the circulation[1]. They originate from pluripotent hematopoietic stem cells via a multi-step differentiation process followed by complex maturation. As MKs mature, they lose the ability to divide but continue to replicate their DNA in a process called endomitosis, resulting in a polyploid and lobulated nucleus that is 16N on average[2]. In addition, they form an elaborate invaginated membrane system and undergo rapid cytoplasmic expansion, leading to the accumulation of a massive amount of cytoplasmic proteins and granules[3]. This huge reorganization process of MKs requires highly regulated signaling pathways in which proteins and lipids as structural components or signaling molecules are intertwined. In platelets, lipids are essential for platelet integrity and play a fundamental role in platelet lifespan, senescence, shape change and aggregation. In a full lipidome analysis of platelets from 2018, our lab identified that roughly 20 % of the lipidome is changing upon platelet activation[4]. We hypothesize that the lipidome of MKs will be converted towards a platelet like lipidome and that the overall lipid amount increases while the cells grow and major rearrangements in membrane structure occur. Using nano-ESI direct infusion combined with high-resolution mass spectrometry in data independent acquisition mode, we were able to identify and quantify almost 300 lipids of the major phospholipid classes and glycerolipids at different time points of MK maturation. A first quantification showed that the overall amount of triacylglycerol (TAG) and phosphatidylglycerol (PG) increases, while the abundance of phosphatidylcholine ether lipids (PC-O) decreases about 7 % over MK maturation. This is already indicating a major membrane remodeling of MKs towards the platelet phenotype. Further ongoing studies will prove which other lipid categories adapt during MK maturation and which transcription factor circuits and enzymatic regulations are mandatory for this reorganization process.

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Quality Control Tools to increase the the Confidence in lipid annotations

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P 39

The field of lipidomics is attracting more and more interest as the essential role of lipids in the emergence and progression of diseases is better understood. Therefore, the need for a standardized and high-quality reporting of lipid annotations is getting more important.

The usage of improved acquisition techniques enhances the coverage and quality of the data from mass analyzers – and with this the total number of compounds that can be annotated. These increased numbers eventually need quality control, ideally automated and graphically clearly presented. Therefore, any tool that can be used to efficiently remove false annotations will simplify the researchers work.

LC-MS/MS data of SRM 1950 and Fibroblast lipid extracts were acquired in positive and negative PASEF mode. All 4D datasets were processed with MetaboScape. The retention time aligned features were listed in a single table. In the process of the feature extraction, all important qualifiers such as accurate masses, isotopic patterns, retention times, MS/MS spectra and CCS values were extracted for all specified adducts and neutral losses. A library free rule-based annotation was applied to the complete feature table and CCS values were predicted automatically. Also, retention time as well as CCS value outliers were determined. The corresponding lipids were manually investigated using 4D Kendrick Mass Defect (KMD) plots for data evaluation.

Goslin 2.0 implements the most recent lipid short-hand nomenclature for MS-derived lipid structures

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P 40

Unifying lipid names is a crucial task for computational lipidomics analyses especially when obtaining lipid lists from different analysis tools or laboratories. Goslin[1] is a grammar-based computational library for the recognition/parsing and normalization of lipid names following the lipid shorthand nomenclature[2]. The new version Goslin 2.0 especially implements the latest nomenclature[3]. An additional grammar was added that recognizes systematic IUPAC fatty acyl names (such as stored, e. g. in the LIPID MAPS database). Main updates include the annotation of ring double bond equivalents instead of double bonds and number of oxygen atoms to facilitate also a hierarchical reporting of oxygenated lipid species. The free fatty acid category was completely updated and now covers fatty acids and conjugates, fatty alcohols, wax monoesters, N-acyl amines, etc. In addition, functional groups were added into the nomenclature, such as the COOH, OOH groups. Enclosed structures like acyl/alkyl branches or cycles can be described now, too. Along with the original shorthand version, the structural level of the provided information is preserved. As an application, Goslin 2.0 is perfectly suited to parse, normalize or update lipid names in databases as LIPID MAPS, Swiss Lipids, or HMDB to support the latest nomenclature. It is already included in tools such as LipidCreator[4] bringing them up to scratch again. With the capability of normalizing more than 1000 lipid names per second, Goslin 2.0 is well suited as a real-time element of a computational lipidomics workflow and outperforms other existing lipid standardization tools. Goslin 2.0 is implemented as a stand-alone web application with a REST API as well as C++, C# (added in Goslin 2.0), Java, R, and Python libraries that can easily be included in lipidomics tools and scripts providing direct access to the translation functions. All implementations are open source.

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Lipi Detective: a Deep Learning Model for the Detection of Lipid Species in Mass Spectra

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P 41

To enable a systems-level understanding of the various lipid functions, innovative techniques have to be explored to optimize, accelerate and automate the identification and quantification of molecular lipid species in biological samples. A fast and accurate identification of molecular lipid species from mass spectra via artificial neural networks would enable researchers to analyze more complex datasets in less time. It could also assist in the estimation of false discovery rates and thereby improve the quality and reliability of data analysis in lipidomics.

The aim of this study was to test the ability of artificial neural networks to identify lipid species based on their characteristic fragmentation patterns in mass spectra. For this purpose, a pilot model called LipiDetective was implemented and trained on a reference dataset containing 49 different phospholipid standards. Since fragmentation patterns change with varying collision energy the lipid standards were measured with 13 different collision energies starting from 20.0 eV up to 50.0 eV and increasing in 2.5 eV increments. The MS2 spectra resulting from these measurements provided the basis for the data set of fragmentation patterns on which the networks were trained.

In the framework of this project the performance of a feedforward as well as a convolutional neural network was evaluated. The output of the model consists of three continuous values corresponding to the predicted masses for the headgroup and two fatty acid chains of the phospholipids. Therefore, the definition of accuracy as it is commonly used in regular classification problems is not well suited in this case and a new measure called closeness was implemented to evaluate how far away the predicted masses are from the true values.

Based on the predictions using the optimal parameter setup, the feedforward network can identify the different phospholipid species with an accuracy of 60 % to 70 %. The convolutional neural network performs similar, but shows less overfitting compared to the feedforward network. Additionally, there are still some further parameters that have to be optimized for the convolutional neural network, which might significantly improve its performance.

In general, the predictions of the network are quite close to the true value, but a higher resolution still needs to be achieved in order to distinguish fatty acids that differ by a single double bond. All in all, the pilot model showed thatusing neural networks for identifying lipid species from fragmentation spectra is generally feasible. It also revealed that further optimization of the network parameters is a crucial factor in enhancing the predictive performance and that increasing the amount of training data is key for a successful application to mass spectra from complex lipidomes.

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