iLS

INTERNATIONAL LIPIDOMICS SOCIETY

LIPIDOMICS
FORUM

8th Lipidomics Forum & 2nd ILS Conference
August 27th - 30th 2023

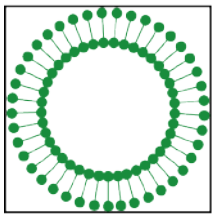# AbstractBook

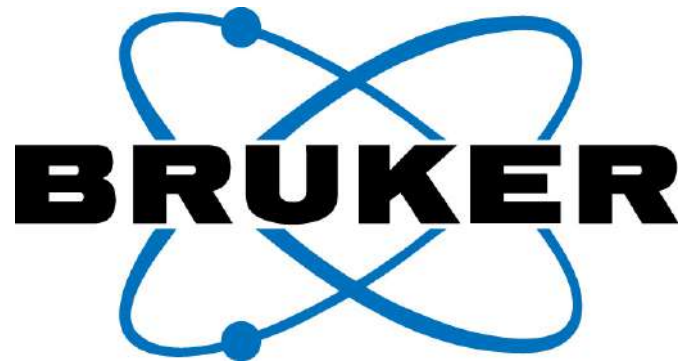**Research Center Borstel**
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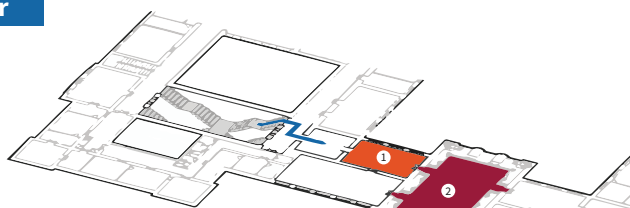
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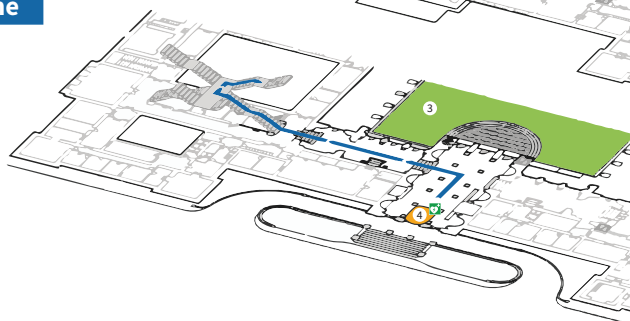Harald Köfeler, Medical University Graz, Graz, Austria

Orientation Plan

University Main Building / Universitätsring 1

1st Floor

Mezzanine

Heurigen Evening by ILS & MANA
Mayer am Pfarrplatz
Pfarrplatz 2, 1190 Vienna

Conference Dinner
Die Schankwirtschaft im Augarten
Obere Augartenstraße 1A, 1020 Vienna

1 Registration & Cloakroom
Via staircase 1

2 Lecture Hall
Main Ceremonial Hall

3 Get Together
Arcaded courtyard

4 Entrance
Universitätsring 1, 1010 Vienna

Faculty of Chemistry / Währinger Straße 38

2nd Floor
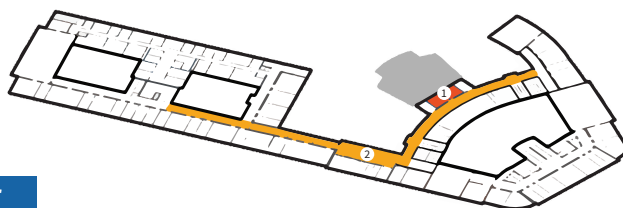
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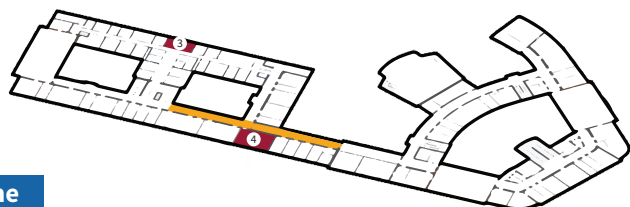
Mezzanine
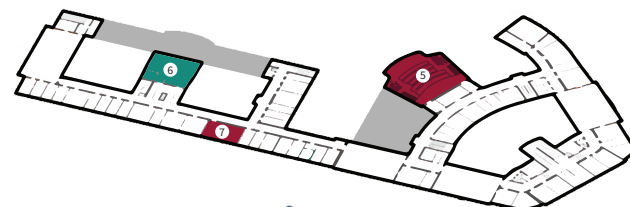
Ground Floor
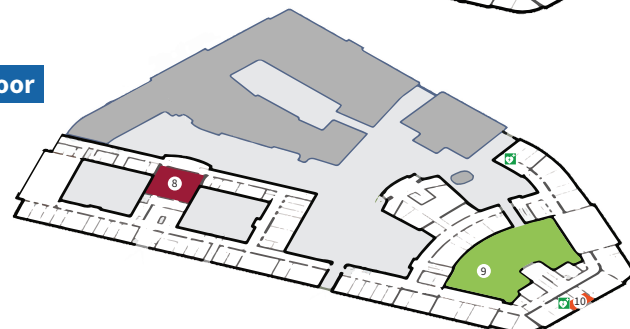
1 Cloakroom
Seminar room 1, room 1247

2 Poster Area
Additional plan for more details

3 LipiTUM Workshop
Intermediate storey, room 2153-W

4 Meeting Microbial Lipidomics
Seminar room 2, room 2124

5 Lecture Hall
Carl Auer von Welsbach lecture hall

6 Vendor Exhibition & Coffee Break
Studierendenzentrum

7 LIFS Bioinformatics Training
"Kleiner Hörsaal 4", room 2H23

8 CLIG Meeting
Joseph Loschmidt lecture hall

9 Lunch Break
Backyard

10 Entrance & Registration
Währinger Straße 38, 1090 Vienna

Schedule

Sunday | **August 27** | Main Building

10:00 – 15:00	**WORKSHOPS | WÄHRINGER STR.**
	LIFS (Room 2H23) | TUM Bioinformatics (Room 2153-W) | Microbial Lipidomics (Room 2124)

15:00 – 16:00	**REGISTRATION OPENING**

16:00 – 16:15	**CONFERENCE WELCOME**
	Manuela Baccarini, Vice-Rector for Research and International Affairs

16:15 – 18:00 **OPENING KEYNOTE** | Chair: Robert Ahrends

Platt, Frances

T01 **Sphingolipid lysosomal storage diseases**

University of Oxford, Oxford, United Kingdom 10

18:00 – 21:00	**GET TOGETHER**
	Acardenhof, Drinks and Snacks

Monday | **August 28** | Währinger Str. 38

8:00 – 9:00	**REGISTRATION**

9:00 - 9:45 **TUTORIAL** | Chair: Kim Ekroos (9:00 – 11:45)

Höring, Marcus

T02 **Do's and Don'ts during the Preanalytical Stage and Extraction of a Lipidomics Workflow**

University Hospital Regensburg, Regensburg, Germany 11

9:45 - 10:30 **KEYNOTE**

D'Angelo, Giovanni

T03 **The lipotype hypothesis**

École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland 12

10:30 – 11:00	**COFFEE BREAK**
	Served in the 'Studierendenzentrum'

11:00 – 11:45 **KEYNOTE**

Fedorova, Maria

T04 **Lipidome dynamics at the crossroad between survival and cell death**

TU Dresden, Dresden, Germany 13

11:45 – 12:45 **SESSION 1 (INFORMATICS)** | Chair: Dominik Kopczynski (11:45 – 12:45)

11:45 **PRIZE** Saylan, Cemil Can

T05 **An Automated Lipid Identification Pipeline for Electron-Activated Dissociation Mass Spectrometry Experiment**

Technical University of Munich, Freising, Germany 14

12:05 Vondráčková, Michaela

T06 **LORA: Lipid Over-Representation Analysis based on structural information**

Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic 15

12:25 Bonini, Paolo

T07 **Predicting liquid chromatography retention times using model ensembling in lipidomics**

oloBion, Barcelona, Spain 16

12:45 – 13:45	**LUNCH**	
	Served in the backyard	
	CLIG Meeting (Joseph Loschmidt lecture hall, hosted by CLIG board)	

13:45 – 14:10	**KEYNOTE** | Chair: Michal Holčapek (13:45 – 16:45)	
	O'Donnell, Valerie B	
T08	**Elucidating the role of phospholipids in abdominal aortic aneurysm**	
	Cardiff University, Cardiff, United Kingdom 17	

14:10 - 15:30	**SESSION 2 (DYNAMICS)**	
14:10	Ghorasaini, Mohan	
T09	**Single workflow for subsequent lipids and lipoproteins measurement in rheumatoid arthritis plasma**	
	Leiden University Medical Center, Leiden, The Netherlands 18	
14:30 **PRIZE**	Rund, Katharina M.	
T10	**Effects of ion suppression and matrix interference on untargeted LC-MS/MS analysis of polar lipids in human plasma**	
	University of Wuppertal, Wuppertal, Germany 19	
14:50	Maschek, J. Alan	
T11	**Comparative Study of Lipidomic Profiles in Fed versus Fasted States and Across Blood Collection Tube Matrices: Implications for Medium to Large-Scale Epidemiological Studies**	
	University of Utah, Utah, United States of America 20	
15:10 **PRIZE**	Shiian, Aleksandra	
T12	**Metabolic lipid tracing in the murine heart**	
	LIMES Institute, Bonn, Germany 21	

15:30 – 16:00	**COFFEE BREAK**	
	Served in the 'Studierendenzentrum'	

16:00 - 16:45	**KEYNOTE**	
	Meikle, Peter J	
T13	**High through put plasma lipidomics: Development and translation of metabolic and cardiovascular risk scores.**	
	Baker Heart and Diabetes Institute, Melbourne, Australia 22	

16:45 – 18:45	**POSTER SESSION – I (ODD POSTER NUMBERS)**	

19:30	**HEURIGEN EVENING by ILS & MANA**	
	Mayer am Pfarrplatz - Pfarrplatz 2, 1190 Vienna	

Tuesday | August 29 | Währinger Str. 38

9:00 - 9:45	**KEYNOTE** | Chair: Cristina Coman (9:00 – 10:30)	
	Thiele, Christoph	
T14	**The dynamics of lipid metabolism - alkyne lipids as sensitive tracers**	
	LIMES Institute, Bonn, Germany 23	

9:45 – 10:30	**KEYNOTE**	
	Sáenz, James	
T15	**Minimal membrane systems for deciphering the role of lipidome complexity**	
	TU Dresden, Dresden, Germany 24	

10:30 – 11:00	**COFFEE BREAK**	
	Served in the 'Studierendenzentrum'	

11:00 – 12:40	**SESSION 3 (STRUCTURE)** | Chair: Maria Fedorova (11:00 – 14:25)

11:00   Salamin, Olivier

T16   **Comprehensive Analysis of Octadecanoid Oxylipins using Chiral SFC-MS/MS and Reversed-Phase-LC-MS/MS Methods**

Karolinska Institutet, Solna, Sweden 25

11:20   Xia, Yu

T17   **Empowering Lipidomic Profiling with Isomer-Resolved Mass Spectrometry**

Tsinghua University, Peking, People's Republic of China 26

11:40   **PRIZE**   Mueller, Patrick

T18   **Determining Stereospecific Numbering and Double Bond Positions of Acylglycerols in Human Plasma using Supercritical Fluid Chromatography Photoionization Mass Spectrometry and Collision-Induced Dissociation of Radical Cations**

University of Geneva, Geneva, Switzerland 27

12:00   Takahashi, Hidenori

T19   **Novel Ion Fragmentation in Mass Spectrometry for Detailed Lipid Structural Analysis via Atomic Hydrogen/Oxygen Irradiation**

Shimadzu Corporation, Kyoto, Japan 28

12:20   **PRIZE**   Kadyrbekova, Yasmin

T20   **Novel derivatization method for the determination of sterols, prenols, and neutral lipids by RP-UHPLC/MS/MS in negative ion mode**

University of Pardubice, Pardubice, Czech Republic 29

12:40 – 13:40	**LUNCH**

Served in the backyard

13:40 – 14:25	**KEYNOTE**

Takats, Zoltan

T21   **n-vivo lipidomics: Towards molecularly-guided autonomous surgical robots**

Imperial College London, London, United Kingdom 30

14:25 – 15:25	**SESSION 4 (MEMBRANE)** | Chair: Dominik Schwudke (14:25 – 15:55)

14:25   John Peter, Arun

T22   **Unveiling the Secrets of Lipid Transport In Vivo using METALIC**

University of Fribourg, Switzerland 31

14:45   **PRIZE**   Yaghmour, Mohamed H.

T23   **Alkyne lipid tracers and the role of MFSD2a for lipid import into the brain**

University of Bonn, Bonn, Germany 32

15:05   Hornemann, Thorsten

T24   **Motor Neuron Disease or Sensory Neuropathy? L-Serine as a modulating factor**

Universtiy Zürich, Zürich, Switzerland 33

15:25 – 15:55	**COFFEE BREAK**

Served in the 'Studierendenzentrum'

15:55 – 17:55	**POSTER SESSION – II (EVEN POSTER NUMBERS)**

19:00	**CONFERENCE DINNER**

Die Schankwirtschaft im Augarten - Obere Augartenstraße 1A, 1020 Vienna

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Wednesday | **August 30** | Währinger Str. 38

9:00 – 10:00	**TUTORIAL** | Chair: Yu Xia (9:00 – 10:45)

Schuhmann, Kai

T25   **Accurate lipid quantification without fragmentation bias**

10:00 – 10:45 **KEYNOTE**

Ivanisevic, Julijana

T26 **Omics-Scale High-Throughput Targeted LC-MS/MS Approach for Lipid Profiling: From Development to Application**

10:45 – 11:15 **COFFEE BREAK**

Served in the 'Studierendenzentrum'

11:15 – 12:55 **SESSION 5 (ANALYTICS)** | Chair: Nicolas Gisch (11:15 – 13:40)

11:15 Barsch, Aiko

T27 **Profiling and quantitation of bile acids in human biofluids by LC-TIMS-MS**

11:35 Dreisewerd, Klaus

T28 **Recent methodological advances of MALDI-2 and t-MALDI-2 mass spectrometry for molecular imaging of lipids**

11:55 Saunders, Kyle

T29 **Optimisation and Application of Single Cell Lipidomics**

12:15 Lehmann, Rainer

T30 **Hidden obstacles in clinical lipidomics –Ex vivo stabilities of lipid species in blood**

12:35 Suh, Jung H.

T31 **Metabolomic Profiling of Plasma Samples from PPMI Identifies Molecular Signatures of Parkinson's Disease and Genetic Parkinson's Disease**

12:55 – 13:40 **KEYNOTE**

Shevchenko, Andrej

T32 **Emerging developments in shotgun lipidomics**

13:40 – 14:10 **CLOSING SESSION** | Chair: Robert Ahrends

Prizes for best Poster and Talk awarded by Lipotype

14:10 **PICKUP LUNCHES**

Served in the backyard

14:10 – 15:30 **DGMS INTEREST GROUP MEETING**

Lipid Analysis and Lipidomics

Abstracts of Talks

Sphingolipid lysosomal storage diseases

Platt, Frances[1]

[1] University of Oxford, Oxford, United Kingdom

T01

Lysosomal storage diseases (LSDs) are a group of rare inborn errors of metabolism that are characterised by the accumulation of macromolecules in the lysosome. Inherited mutations in genes encoding lysosomal enzymes account for the majority of these diseases, with other lysosomal disorders arising from defects in genes encoding lysosomal membrane proteins or proteins that affect lysosomal function through other mechanisms.

There are many LSDs that involve lipid storage. In this presentation, I will focus on the primary and secondary sphingolipidoses. I will then go into detail on the remarkable complexity of a single monogenic lysosomal sphingolipid storage disease, Niemann-Pick disease type C (NPC). I will discuss how the study of this rare neurodegenerative disorder links to disparate common and rare diseases, and explain how this disease is shedding light on unanticipated aspects of cell biology. I will end by discussing how the knowledge gained from this research is being translated into therapies for NPC and related diseases.

Do's and Don'ts during the Preanalytical Stage and Extraction of a Lipidomics Workflow

Höring, Marcus[1], Stieglmeier, Christoph[1], Liebisch, Gerhard[1]

[1] University Hospital Regensburg, Regensburg, Germany

T02

Lipidomics is a dynamic and rapidly evolving field that aims to comprehensively study lipid molecules in cells, tissues, and biofluids. The term 'preanalytics' (or preanalytical conditions) refers to a set of procedures and considerations involved in sample collection, handling, storage, and preparation prior to lipid analysis. These steps are crucial, as lipid molecules derived from biological sources are susceptible to degradation, primarily attributed to oxidation and enzymatic hydrolysis as the main contributing factors. The heterogeneity of sample materials presents further challenges. While the handling of biofluid samples is generally straightforward, the required homogenization of tissues necessitates further considerations. Another key element of lipidomics analysis is the extraction of lipid molecules. Some of these protocols, such as the procedure described by Bligh and Dyer or the Folch extraction, have been in use for over 60 years. However, numerous extractions have been developed and refined in recent decades. Nevertheless, suitable extraction methods need to be efficient, selective, and reproducible.

Implementing standardized procedures for these steps is essential for maintaining sample integrity, minimizing contaminations, and preserving the physiological state of the samples to obtain high-quality lipid extracts. This tutorial will discuss potential pitfalls during the preanalytical stage and extraction of a typical lipidomics workflow.

The lipotype hypothesis

D'Angelo, Giovanni[1]

[1] École Polytechnique Fédérale de Lausanne, Lausanne, Switzerland

T03

Single-cell genomics techniques have allowed for the deep profiling of individual cells in multicellular contexts. These new technologies have enabled the building of cell atlases where hundreds of different cell types are categorised according to their transcriptional and epigenetic states. These analyses have led to the depiction of detailed cell transcriptional landscapes that could be interpreted in terms of cell identity. Nonetheless, transcription represents only one axis in the establishment of cell phenotypes and functions and post-transcriptional events crucially concur to cell identity in ways that cannot be simply derived from transcriptional profiles. Thus, the chemical composition of individual cells and the activity of metabolic pathways are likely as good descriptors of cell identity as transcriptional profiles are. Moreover, accumulating findings assign to lipid metabolism an instructive role towards the establishment of cell identity, yet our understanding of the integration of transcriptional and lipid metabolic programs in cell fate determination remains superficial. Here I will report on our attempts to investigate lipidomes at single cell levels and at high spatial resolution by MALDI imaging mass spectrometry.

Lipidome dynamics at the crossroad between survival and cell death

Wölk, Michele[1], **Fedorova, Maria**[1]

[1] TU Dresden, Dresden, Germany

T04

Maintaining homeostasis is one of the key features of cellular and organismal organization. The mechanism behind homeostatic control at molecular level involve dedicated machineries overseeing DNA, protein and lipid qualities. In contrast to DNA and proteins, lipid quality control (LQC) is so far poorly understood and represents an emerging topic. The regulation of the cellular lipid homeostasis in response to various stimuli is important to maintain the physiological status of a cell and to allow adaptation to everchanging environment. Among numerous stimuli cells can experience during their life cycle is redox stress. Lipids are especially sensitive to variations in the levels of reactive oxygen species due to the large number of polyunsaturated acyl chains which can be easily oxidized leading ultimately to the membrane rupture and cell death. Redox stress was studied on the multiple levels with a large set of proteins identified to be responsible for cell survival or death. However, the details mechanism behind lipidome remodelling in pro-oxidative conditions are much less studied. Here we used high resolution LC-MS/MS based lipidomics to address adaptive vs lethal responses of cellular lipidome in pro-oxidative conditions in time resolved manner. We could identify specific lipid signatures up- or down-regulated upon cell death propagation and/or adaptation. For instance, specific sub-classes of ceramide lipids showed strong upregulation upon redox disbalance. Using a combination of subcellular fractionation, lipidomics and machine learning approaches we are aiming to reveal a detailed time-resolved image on subcellular lipidome remodelling under oxidative stress conditions to promote a better understanding on the lipid quality control machinery.

An Automated Lipid Identification Pipeline for Electron-Activated Dissociation Mass Spectrometry Experiment

Saylan, Cemil Can[1], Köhler, Nikolai[1], Haubs, Ferdinand[1], Koller, Franziska[1], Schwartz, Leon[1], Nahrstedt, Vivienne[1], Stockhaus, Sonja[1], Pauling, Josch K.[1]

[1] Technical University of Munich, Freising, Germany

T05 PRIZE

Lipids are crucial components in various biological processes. Detailed information about the exact structure of lipids is necessary for comprehending their functions and significance in diverse biological processes. Over the last few decades, the lipid research area has rapidly expanded. Lipid analysis using Mass Spectrometry (MS) is a common technique. However, the current methods are limited in the structural elucidation of lipids.

A recently introduced method known as electron-activated dissociation (EAD) has emerged as a valuable technique for the structural elucidation of lipids. EAD is capable of generating different fragmentation patterns for closely related lipid species, facilitating their precise identification. Unlike other MS techniques, EAD can characterize acyl chains, leading to a series of fragment ions that can pinpoint the location of double bonds. Identification includes the position of double bonds for each fatty acid and the sn-positions for each lipid species. The increasing popularity of EAD for the identification and characterization of lipids highlights the need for efficient computational tools.

This project aims to develop an open-source lipid species identification tool to provide an efficient way to analyze EAD-based mass spectrometry experiments. Lipid candidates for each MS2 spectrum are identified using exact precursor mass matching and head group fragment identification. The identification of the molecular formula of lipids is computed via two scoring models. The first scoring approach is rules-based in which the measured peaks for a given tolerance range for lipid class and sn-positions are evaluated against generated reference spectra based on their m/z value similarities. The reference peaks used in the rules-based model are created for the candidate lipids' head group, sn, and fatty acid fragments according to expected m/z values. The second method is generated using the Fourier-transform (frequency-based) to resolve the double-bond positions of a given MS2 spectrum. The groups of peaks for the fatty acid chain fragments are represented as frequencies. Then, the double bond positions are identified based on changes in the frequency.

The obtained preliminary results show that the combination of the rules-based approach for general lipid species identification and the Fourier-transform frequency-based method for identification of double-bond positions on each hydrocarbon chain achieve confident results despite a general lack of optimization at this point of development. This is the first reported automated identification workflow for EAD mass spectrometry lipid data that will facilitate the next step in lipid structural identification. This will, in turn, fuel investigations of the roles of lipids in metabolism and the unraveling of (patho-) mechanisms increasing the relevance of lipidomics in clinical research.

LORA: Lipid Over-Representation Analysis based on structural information

Vondráčková, Michaela[1], Kopczynski, Dominik[2], Hoffmann, Nils[3], Kuda, Ondřej[1]

[1] Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

[2] Institute of Analytical Chemistry, University of Vienna, Austria

[3] Forschungszentrum Jülich, Institute of Bio- and Geosciences (IBG-5), Germany

T06

With the increasing number of lipidomic studies, there is a need for efficient and automated analysis of lipidomic data. One of the challenges faced by most existing approaches to lipidomic data analysis is lipid nomenclature. The systematic nomenclature of lipids contains all available information about the molecule, including its hierarchical representation, which can be used for statistical evaluation. The Lipid Over-Representation Analysis (LORA) web application (https://lora.metabolomics.fgu.cas.cz) analyzes this information using the Java-based Goslin framework, which translates lipid names into a standardized nomenclature. Goslin provides the level of lipid hierarchy, including information on headgroups, acyl chains, and their modifications, up to the 'complete structure' level. LORA allows the user to upload the experimental query and universe datasets, select a grammar for lipid name normalization, and then process the data. The user can then interactively explore the results and perform lipid overrepresentation analysis based on selected criteria. The results are graphically visualized according to the lipidome hierarchy. The lipids present in the most over-represented terms (lipids with the highest number of enriched shared structural features) are defined as Very Important Lipids (VILs). For example, the main result of a demo dataset is the information that the query is significantly enriched with 'glycerophospholipids' containing 'acyl 20:4' at '*sn*-2 position'. These terms define a set of VILs (e.g., PC 18:2/20:4;O and PE 16:0/20:4(5,8,10,14);OH). All results, graphs, and visualizations are summarized in a report. LORA is a tool focused on the smart mining of epilipidomics datasets to facilitate their interpretation at the molecular level.

Predicting liquid chromatography retention times using model ensembling in lipidomics

Bonini, Paolo[1], MEHTA, SAJJAN SINGH[1], BENABOU, SANAE[1], KIND, TOBIAS[2], FIEHN, OLIVER[2]

[1] oloBion, Barcelona, Spain

[2] West Coast Metabolomics Center (UC Davis Genome Center), Davis, USA

T07

Untargeted lipidomics yields hundreds of unique small molecule signals. Exact mass tandem mass spectra are generally considered insufficient as a sole source for compound annotations as they can match high numbers of false positive candidates, especially when using large spectral libraries. Confident identification workflows require additional orthogonal information such as retention times, but the number of authentic reference compounds with validated experimental retention times are very limited for each chromatographic method. Hence, retention times need to be predicted from chemical structures using the available experimental data to limit false positive annotations.

In this study, Retip 2 applies automated machine learning (AutoML) methods to build ensemble models using a training set of 250 lipids as well as a minimal set of 69 compounds from a commercially-available internal standard mix. With mean absolute errors of ~7.5 seconds and ~20 seconds, respectively, for a 16-minute chromatographic method, this approach delivers high-accuracy retention time prediction and additionally proves to be effective when using very small training sets. Retip 2 was shown to not only improve the predictive performance of Retip 1[1] but also outperform other novel approaches to retention time prediction such as graph neural networks. Additionally, Retip 2 provides interactive notebooks to facilitate model building and usage for non-programmers.

To validate the efficacy of this method, lipidomics analyses standard reference material for human plasma and an internal standard mix were run on three different chromatographic systems. Retention time prediction models were applied during mass spectral identification to allow for scoring based on similarity between the measured and predicted retention times. Filtering using predicted retention times was shown to be highly effective at eliminating false lipid annotations and at highlighting in-source fragmentations. Nearly 30% of top-hit matches scored only using accurate mass and tandem mass spectral similarity were found to be incorrect annotations when utilizing predicted retention time as a simple filter with a cutoff corresponding to the 95% confidence interval of the model.

References

1. Bonini, P., Kind, T., Tsugawa, H., Barupal, D. K. & Fiehn, O. Retip: Retention Time Prediction for Compound Annotation in Untargeted Metabolomics. *Anal. Chem.* **92**, 7515–7522 (2020).

Elucidating the role of phospholipids in abdominal aortic aneurysm

O'Donnell, Valerie B[1], Costa, Daniela O[1], Morgan, Bethan[1], Tyrrell, Victoria J[1], Allen-Redpath, Keith[1], Jenkins, P Vince[1], Mallat, Ziad[2], Lee, Regent[3], Omidvar, Nader[1], Collins, Peter W[1]

[1] Cardiff University, Cardiff, United Kingdom
[2] Dept of Medicine, University of Cambridge University
[3] Nuffield Department of Surgical Services, Oxford University

T08

Over the last 15 years, our research has focused on identification and characterisation of enzymatically-oxidized phospholipids (eoxPL) generated by platelets and leukocytes. We found that these are pro-coagulant in vitro and in vivo, through supporting PS to bind and activate circulating factors at membrane surfaces. Our current studies in humans and murine models aim to determine the generation and action of these lipids in abdominal aortic aneurysm (AAA), a disorder where the aorta develops an inflammatory thrombotic lesion which is at risk of sudden devastating rupture.

Mice lacking either 12/15-LOX (leukocytes) or 12-LOX (platelets) were found to be protected against AAA in the angII/ApoE$^{-/-}$ model. Numerous eoxPL were detected in AAA lesions from wild type mice and the disease was associated with coagulopathy. Lesion development could be prevented using a FX inhibitor, showing that coagulation is causally driving disease. eoxPL were also detected in human AAA lesions. Currently, the role of eoxPL in elastase-induced AAA, which involves local damage to the aorta, instead of the systemic inflammatory challenge of angII/ApoE$^{-/-}$ is being characterised. Preliminary data shows that this model maybe less dependent on both coagulation and eoxPL. In a human AAA cohort, eoxPL and native phospholipids (PL) are being profiled in plasma and AAA tissue, to examine which lipid species may be associated with altered coagulation. When stratifying AAA subjects genetically via a known AAA risk SNP in Chr9p21, many correlations in plasma PL metabolism are seen for the risk SNP that are completely reversed in the non-risk SNP. Further studies aim to understand the underlying biochemical changes responsible. In this presentation, recent data on both the mouse model and human cohort will be presented.

Single workflow for subsequent lipids and lipoproteins measurement in rheumatoid arthritis plasma

Ghorasaini, Mohan[1], Tsezou, Konstantina Ismini[2], Verhoeven, Aswin[1], Mohammed, Yassene[1,3], Vlachoyiannopoulos, Panayiotis G.[2,5], Mikros, Emmanuel[2,4], Giera, Martin[1]

[1] Leiden University Medical Center, Leiden, The Netherlands

[2] Department of Pathophysiology, School of Medicine, National and Kapodistrian University of Athens, 157 71 Athens, Greece

[3] Genome BC Proteomics Centre, University of Victoria, Victoria, BC V8Z 5N3, Canada

[4] Pharmagnose S.A., 320 11 Inofyta, Greece

[5] Institute for Autoimmune Systemic and Neurologic Diseases, 104 31 Athens, Greece

T09

Lipids are transported throughout the human body by lipoproteins. Lipoproteins are droplets of mostly lipids and, to a lesser extent, proteins that allow hydrophobic lipids to participate in circulation. The lipid composition of lipoprotein particles is determinative of their respective formation and function. Therefore, combination of Nuclear Magnetic Resonance (NMR)-based lipoprotein measurements with Mass Spectrometry (MS)-based lipidomics is an appealing technological combination for a better understanding of lipid metabolism in health and diseases.

In this work, we developed a combined workflow for subsequent NMR- and MS-based analysis on single sample aliquots of human plasma and evaluated the congruence and complementarity between the platforms in order to facilitate a better understanding of patho-physiological lipoprotein-lipid alteration. Utilizing the non-destructive sample analysis by NMR, firstly, we performed lipoprotein measurement followed by lipid measurement by MS on single sample aliquot. We compared lipid class concentrations between healthy controls and rheumatoid arthritis (RA) patient samples to investigate consensus among the platforms on differentiating two groups. Next, we performed correlation analysis between all measured lipoprotein particles and lipid species. Additionally, we generated correlation heatmaps detailing lipoprotein/lipid interactions and describe disease-relevant correlations.

We found excellent agreement and correlation (r>0.8) between the platforms for lipid class quantification and differentiation of healthy control and RA plasma materials. Furthermore, we confirmed the known correlations such as very low density lipoprotein-triglycerides (VLDL-TG) and more and present a rich dataset for further data mining. To further substantiate the value of this analysis, we present potential novel biomarker candidates based on lipoprotein–lipid species correlation. Although biomarker discovery and validation will demand additional work, we show here that lipoprotein–lipid species correlation might serve as potential biomarkers and grant novel insights into pathophysiological mechanism. In summary, combined NMR/MS analysis is feasible and provides added value when compared with the individual platforms.

Effects of ion suppression and matrix interference on untargeted LC-MS/MS analysis of polar lipids in human plasma

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T10 **PRIZE**

Lipids are essential molecules involved in a wide range of biological functions including energy metabolism, cell signaling, regulation of inflammation and cell membrane assembly. Driven by the biological importance of lipids, lipidomics has emerged as a major field of research in the past decades.

Liquid chromatography (LC) coupled by electrospray ionization (ESI) to high-resolution mass spectrometry (HRMS) is a powerful approach as it can monitor simultaneously a broad variety of lipids extracted from biological samples. However, the highly complex matrix of these samples can cause interference such as ion suppression in ESI.

Here, we investigate how matrix effects affect untargeted LC-HRMS/MS (orbitrap) analysis: Extraction efficacy of lipids from plasma was investigated using a set of isotopically labeled internal standards (IS, SPLASH mixture). Moreover, by constant post-column infusion of the internal standards, ion suppression was investigated in detail.

Lipids were extracted by liquid-liquid extraction with methyl *tert*-butyl ether and methanol. The chromatographic separation was performed on a C18 reversed-phase column (100 x 2.1 mm, 1.7 µm). The ionization was carried out by ESI using a heated electrospray HESI-II in positive and negative modes. Mass spectrometric detection was achieved using Full MS/data-dependent MS^2 with a resolution set to 60,000 for full MS by means of a hybrid quadrupole-orbitrap (Thermo Scientific Q Exactive HF).

Spiking experiments of IS prior and post extraction revealed overall excellent extraction efficiency (> 85%) for isotopically labeled IS. Ion suppression analysis by post-column infusing unveiled that apparent losses are due to ion suppression of coeluting lipids. Especially in positive mode, ion suppression was observed. The ion suppression analysis also unveiled that the signal of two internal standards (PI 15:0/18:1[D7], PG 15:0/18:1[D7]) dropped to zero over several minutes of the retention time. We show that this is not caused by ion suppression but by interfering matrix compounds with a similar *m/z*. Here, the orbitrap Fourier transformation or the software assigned the signals of the two ions to only one *m/z* in the matrix at the selected resolution of 60,000. On the poster we highlight that signals of ions can be erased by orbitrap measurements as a cautionary note, emphasizing that the choice of the resolution as well as careful data analysis of LC-MS/MS is important for untargeted lipidomics.

Comparative Study of Lipidomic Profiles in Fed versus Fasted States and Across Blood Collection Tube Matrices: Implications for Medium to Large-Scale Epidemiological Studies

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T11

High throughput measurement of blood lipids can provide valuable insight the role of lipid metabolism in human health and disease. Bioactive sphingolipids like ceramides are key regulators of cellular processes and metabolic homeostasis and are associated with risk of metabolic diseases like diabetes, cardiovascular disease, and cancer. The metabolic state, particularly fasting or feeding, influences lipid metabolism, but whether fasting state influences concentration of specific bioactive sphingolipids remains unclear. Epidemiologic studies typically store participant serum, plasma, or both, and plasma anticoagulant used in blood collection tubes can differ across studies. Yet, it is unknown whether blood tube matrix affects lipid concentration. This study investigated the variation in concentration of a targeted panel of 60 sphingolipids from plasma and serum among individuals in fed and fasted states, and differences in lipid concentration according to blood collection tube matrix.

A cohort of 26 healthy participants aged ≥18 years was recruited. Average age was 37±11, and 42% were female. BMI was 26±7, on average. We collected blood from each participant in both fed and fasted states following standardized protocols. Participants followed specific dietary guidelines to ensure consistency in nutritional intake prior to sample collection. Following an overnight fast, participants had an initial blood draw and were then fed breakfast after which a second blood draw was conducted 90 minutes later. Blood was collected in the following tubes: serum, plasma EDTA, plasma sodium citrate, plasma heparin, and plasma lithium citrate. Liquid chromatography-tandem mass spectrometry (LC-MS/MS) in both positive and negative ionization modes analyzes the lipid profiles. Single-phase and dual phase liquid extractions were evaluated. Statistical analyses and multivariate modeling techniques were employed to compare lipid concentration between the fed and fasted states, and between blood tube matrices.

The observed differences will inform the design of studies moving forward that measure bioactive lipids, establish best practices for sample collection and facilitate interpretation of findings across studies.

Metabolic lipid tracing in the murine heart

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T12 PRIZE

The heart is a highly oxidative tissue and needs to constantly generate ATP to maintain its contractile function, basal metabolic processes and ionic homeostasis. In the adult heart the constant high demand for energy to sustain the continuous myocardial contraction is primarily supported by β-oxidation of fatty acids. On the contrary, the fetal heart relies mostly on glycolysis for the energy production. The metabolic switch from glycolysis to oxidative metabolism occurs shortly after birth and is connected to the loss of regeneration ability of cardiomyocytes, creating a challenge for heart therapies. While normal myocardial function is highly dependent on a fine-tuned equilibrium between produced and utilized energy, close relationship between altered myocardial metabolism and heart failure has been established. Disruptions in energy homeostasis and, particularly, in fatty acid uptake and metabolism are commonly observed in many forms of heart disease, including heart failure, ischemic heart disease and diabetic cardiomyopathies. However, pathogenesis of these changes in impaired hearts is incompletely understood. Thus, understanding changes in fatty acid uptake and metabolism in developing hearts as well as lipid dysregulation mechanisms in adult hearts is of high medical importance.

This project aims to explore the difference in uptake and metabolism of fatty acids in the murine heart in respect to their chain length and saturation degree. In our research we intend to apply alkyne-fatty acid tracers to the murine hearts using different *in vivo* and *in vitro* setups. After alkyne-fatty acids incorporation into cellular lipids, the click-reaction is performed and an azide-tag with specific mass and fragmentation pattern is attached to the alkyne bond of newly synthesized lipids, allowing to determine the identity of labelled lipid species via mass spectrometry. The initial results of metabolic lipid tracing in the murine heart will be presented and discussed.

High through put plasma lipidomics: Development and translation of metabolic and cardiovascular risk scores.

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T13

Dysregulation of lipid metabolism underpins multiple diseases, including type 2 diabetes (T2D), cardiovascular disease and age-related dementia. While the metabolic pathways of lipids are well characterised, dysregulation of the same pathways due to environmental and genetic influences is not well understood. We have developed a high throughput lipidomics platform that can analyse over 750 individual lipid species from 10 µL plasma in 15 minutes. Application of this platform to large clinical and population studies is providing new insight into the role of lipid metabolism in chronic disease, new markers for risk assessment and the opportunity for integration with genomic datasets for a systems approach to identify causal pathways with the potential for therapeutic intervention.

Our recent analyses of two large population cohorts; the Australian Diabetes, Obesity and Lifestyle Study (AusDiab, n=10,300) and the Busselton Health Study (BHS, n=4,492), have provided an opportunity to investigate new concepts in population lipidomics. These include the use ridge and LASSO regression models for the development of metabolic BMI and metabolic AGE scores, that can provide new, easily interpretable, measures of metabolic health. Using an analogous strategy, we have developed a lipidomic risk score (LRS) for cardiovascular disease by using the lipidome (predictor) to model the Framingham risk score (FRS, outcome). The LRS provided an improvement in AUC of 0.11 (95% CI 0.04-0.19) and 0.08 (95% CI 0.01-0.14) in the intermediate-risk group ($0.1 \leq$ calibrated FRS < 0.2) in AusDiab (cross-validation) and BHS (external validation), respectively. In AusDiab, the LRS yielded a continuous net reclassification improvement (NRI) of 0.20 (95% CI 0.01-0.39) among cases and 0.19 (95% CI 0.11-0.28) among non-cases. In BHS, the NRI was 0.25 (95% CI 0.03-0.46) among cases and 0.12 (95% CI 0.03-0.22) among non-cases.

These data demonstrate the potential of this approach for the development and translation of lipidomic based scores for metabolic health and disease risk.

The dynamics of lipid metabolism - alkyne lipids as sensitive tracers

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T14

Fatty acids are rapidly taken up by cells and are esterified into hundreds of phospholipid and neutral lipid species. These initial products can subsequently be remodeled and the fatty acids themselves be elongated, desaturated or otherwise modified. We have recently developed a tracing technology that is based on alkyne fatty acids and a set of click reporter molecules optimized for MS detection (Thiele et al., Nature Methods 2019) and applied this to analysis of lipid metabolism in hepatocytes (Wunderling et al., Mol. Metab. 2021) and triglyceride cycling in adipocytes (Wunderling et al., Nat. Metab. 2023). Current works aims at widening the scope to other lipid classes, e.g. ether lipids and sphingolipids and to other cells, tissues and organs. The talk will show recent technological progress and novel directions of applications.

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Minimal membrane systems for deciphering the role of lipidome complexity

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T15

There is no life without lipids. Lipids are essential for cell-environment interactions, cellular organization, and could have been instrumental in the emergence of life itself. Despite significant evolutionary divergence in the molecular composition of biological membranes, all known life forms converge on a single strategy for building responsive interfaces – the lipid membrane. Although a membrane can be assembled from only one type of lipid, biological membranes are built from a staggering diversity of lipid species. Why have cells evolved to make membranes that are so diverse and how does life employ the collective properties of lipids to build responsive organizational interfaces? We have developed the Minimal Cell (JCVI-Syn3B) and the bacterial pathogen Mycoplasma mycoides as experimental platforms in which lipidome size can be manipulated. By tuning lipidome size from fewer than 10 to more than 100 lipid species we are beginning to decipher the role of lipidome complexity for cellular fitness.

Comprehensive Analysis of Octadecanoid Oxylipins using Chiral SFC-MS/MS and Reversed-Phase-LC-MS/MS Methods

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T16

Octadecanoids are a subclass of oxylipins that are formed from 18-carbon fatty acids mainly by both enzymatic and radical-mediated oxidation. Although these compounds have long been regarded as unimportant molecules that are 'just fat', some octadecanoids have been found to play crucial roles in multiple biological and physiological processes including inflammation, and metabolic or immune regulation. We recently developed a platform that employs chiral separation by supercritical fluid chromatography (SFC) and reversed-phase liquid chromatography (LC) hyphenated to tandem-mass spectrometry (MS) to quantify a wide spectrum of octadecanoids. To increase the availability of analytical standards, we synthesized >80 compounds in-house as well as worked with commercial suppliers. The chiral separation is important because the enzymatic biosynthesis of octadecanoids produces compounds with distinct stereochemical configuration, providing useful information on the synthetic route. We have expanded the platform with inclusion of terminal CYP450 metabolites (ω- and ω-1) of linoleic (LA), α-linolenic (ALA) and γ-linolenic acids (GLA) as well as mono-hydroxylated metabolites of ALA, GLA and stearidonic acid (SDA). Using a Waters Trefoil AMY1 column (3.0×150 mm, 2.5 µm), chiral SFC was able to separate 150 octadecanoids, including complex enantioseparations, in <13 minutes with MeOH:EtOH (8:2) and CH_3COOH 0.1% v/v as mobile phase B (mobile phase A being supercritical CO_2) and MeOH and CH_3COONH_4 (5 mM) as the make-up solvent. To improve signal intensity and sensitivity, a gradient based on the co-solvent was utilized for the make-up solvent instead of the initial isocratic flow, thereby preventing excessive use of organic solvent at the source. The achiral LC method employing a ACQUITY Premier BEH C18 column (2.1x100 mm, 1.7 µm) with VanGuard FIT separated 101 species in 12 minutes with mobile phase A consisting of Milli-Q water and CH_3COOH 0.1% v/v and mobile phase B consisting of ACN:IPA (9:1). The use of a shorter column provided better resolution, reduced the flow rate, the run-time, and the overall system pressure. The methods were applied to cnidarian-dinoflagellate symbiosis samples to investigate the role of octadecanoids in coral-symbiosis establishment. It was observed that most of the upregulated octadecanoids "in symbiosis" from both host and symbionts were derived from ω-3 precursors (ALA and SDA). In particular, major differences between some enantiomers were detected suggesting distinct enzymatic pathways in the symbionts. Notably, 12(*R*)-hydroxy-octadecatetraenoic acid (HOTE) was upregulated in symbiont species in symbiotic state and 10-fold more abundant than 12(*S*)-HOTE with an enantiomeric excess (ee) of 85%. Conversely, despite exhibiting a 99% ee in culture, the abundance of 13(*S*)-HOTE was suppressed upon contact with the host. In contrast, the 13(*R*)-HOTE showed a 7-fold increase. This platform provides the most comprehensive method to date for quantifying octadecanoids, enabling the investigation of their biological activity and expanding our knowledge of these bioactive lipids.

Empowering Lipidomic Profiling with Isomer-Resolved Mass Spectrometry

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T17

Structural lipidomics has emerged as a fast-developing research field which is largely supported by the development of isomer-resolved tandem mass spectrometry (MS/MS) methods. Among them, the Paternò-Büchi (PB) derivatization coupled with MS/MS, termed PB-MS/MS, has been demonstrated powerful for the discovery of altered lipid desaturation metabolism and disease phenotyping. Due to the lack of readily ionizable functional groups and structural complexity, profiling of glycosphingolipids (GSLs) suffers from severe matrix effects from phospholipids by conventional liquid chromatography – tandem mass spectrometry (LC-MS/MS). We have established a workflow which integrates magnetic TiO2 nanoparticle-based selective enrichment, offline charged-PB reaction, and LC-MS/MS for profiling of GSLs in brain tissue samples. Identification of GSLs is achieved at multiple structural levels, including chain composition, 2-OH on N-Acyl chain, and C=C location at high sensitivity (sub-nM). This workflow reveals more than 300 distinct GSL molecules in porcine brain, encompassing 5 different headgroups, 4 types of long chain base, and multiple types of C=C location isomers. In-depth profiling of GSLs further enables phenotyping of normal, IDH-wild, and IDH-mutated human brain glioma samples.

Determining Stereospecific Numbering and Double Bond Positions of Acylglycerols in Human Plasma using Supercritical Fluid Chromatography Photoionization Mass Spectrometry and Collision-Induced Dissociation of Radical Cations

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T18 **PRIZE**

Introduction
With the introduction of mass spectrometry (MS) platforms utilizing ultraviolet photodissociation (UVPD) and electron activated dissociation (EAD), radical-driven fragmentation reactions gained substantial attention and became essential tools for the structural elucidation of biomolecules with features inaccessible through collision-induced dissociation (CID) of even-electron precursor ions. While the CID fragmentation of odd-electron radical cation precursors, often observed in EAD and UVPD, has been shown to access orthogonal structural features, little effort was made to push radical cation forming ionization techniques such as dopant-assisted atmospheric pressure photoionization (APPI). We recently demonstrated that, the formation of radical cations can be favored by decoupling chromatographic and ionization conditions using µLC-APPI with post-column addition of methanol. Further, radical cation CID of 40 analytes, including steroids, isoprenoids and polyketides generated spectra similar to electron ionization (EI) which opens the use of EI library searches. In the present work we demonstrate that supercritical fluid chromatography (SFC) coupled to APPI-MS allows to form intact acylglycerol radical cations and to fragment them by CID, which can be utilized for rule-based de-novo annotation of double bond positions and stereospecific numbering (Sn) of acylglycerols in biological samples.

Methods
The lipid fraction of NIST SRM 1950 plasma was extracted using MTBE extraction. SFC was performed using a Nexera UC system (Shimadzu, Kyoto, Japan). Acylglycerols were separated on a Viridis HSS C18 SB Column (Waters, 100Å, 1.8 µm, 3 mm X 100 mm) and analyzed on a TripleTOF 6600 (SCIEX, Concord, ON, Canada) equipped with an APPI source (PhotoSpray, SCIEX, Concord, ON, Canada) using chlorobenzene as dopant or a Turbo V ion source with an ESI or APCI probe (SCIEX, Concord, ON, Canada). CID was performed utilizing data-independent acquisition. EAD spectra were acquired on a ZenoTOF 7600 System (SCIEX, Concord, ON, Canada).

Results
SFC-APPI-MS allows the separation of acylglycerols and subsequent formation of intact acylglycerol radical cations. The signal response of formed radical cations is higher than for ammonia adducts formed by SFC-APCI and comparable to ammonia adduct intensities formed using SFC-ESI. Moreover, CID fragmentation of homogenous and heterogeneous acylglycerol radical cations yield characteristic fragmentation patterns which allow to differentiate between Sn-positions of attached fatty acids and corresponding double bond positions. Observed fragmentation patterns were rationalized and compared to the fragmentation pattern of acylglycerols subjected to EAD. Based on the rational fragmentation of radical cation acylglycerols, a rule-based de-novo lipid annotation software has been developed in R and applied to the analysis of acylglycerols in human NIST SRM 1950 plasma. Conclusively, SFC-APPI-MS/MS offers a powerful alternative to EAD fragmentation for the structural elucidation of acylglycerols in biological samples.

Novel Ion Fragmentation in Mass Spectrometry for Detailed Lipid Structural Analysis via Atomic Hydrogen/Oxygen Irradiation

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T19

In recent years, numerous novel LC-MS methods have been developed for detailed structural analysis of lipids with diverse structures. These methods can be categorized into two groups: those utilizing new fragmentation techniques such as EIEIO (Electron Impact Excitation of Ions from Organics) and UVPD (Ultraviolet Photodissociation), and those applying derivatization using methods like MELDI (mCPBA Epoxidation for Lipid Double-bond Identification) and Paternò-Büchi reactions.

In this study, we have successfully developed a novel radical-induced ion fragmentation utilizing atomic hydrogen (hydrogen abstract dissociation, HAD) and atomic oxygen (oxygen attachment dissociation, OAD) for the first time [1,2]. Atomic hydrogen and atomic oxygen are generated via microwave discharge of water vapor. The generated radicals were introduced into the quadrupole ion trap to initiate HAD/OAD of the target precursor ions. When HAD/OAD is applied to lipid analysis, irradiation of the precursor ions with hydrogen atoms (HAD) causes sequential cleavage of C-C bonds in fatty acids, while irradiation with oxygen atoms (OAD) selectively cleaves C=C bonds. The unique advantage of this technique is its ability to switch between HAD and OAD within a single instrument, enabling the acquisition of structure-dependent information for lipid analysis. Unlike EIEIO, which utilizes electrons, this technique utilizes charge-neutral radical atoms and can be applied in both negative and positive ion modes.

HAD provides valuable information about modification positions, such as OH groups in fatty acids, while OAD is effective in determining the structure of C=C positions. By leveraging the complementary nature of HAD and OAD, we have successfully determined the structures of unique novel metabolites referred to as "goadvionin" which are hybridized compounds of fatty acids and peptides [Onaka et al., Nat. Chem. 12, 869–877, 2020]. These structures could not be resolved using conventional high-resolution mass spectrometry or NMR techniques. Additionally, HAD has contributed to the structure determination of novel biomarkers of Niemann-Pick disease type-C, which remained elusive using conventional mass spectrometry [Maekawa et al., Int J Mol Sci. 2019 Oct; 20(20): 5018].

Furthermore, OAD has been applied to untargeted lipidomics, as demonstrated by Arida and Tsugawa et al., and an analysis platform, including software (MS-DIAL), has been developed [Communications Chemistry, 5-162, 2022]. Through the analysis of human and mice-derived samples, a total of 648 unique lipids, spanning 24 lipid subclasses, were characterized based on their C=C position-resolved information.

In this presentation, we will provide an overview of the mechanism behind this technique and showcase examples of lipid structural analysis using a direct ionization method.

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Novel derivatization method for the determination of sterols, prenols, and neutral lipids by RP-UHPLC/MS/MS in negative ion mode

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T20 **PRIZE**

Lipidomics studies the structure, function, and metabolism of lipids. Lipids are essential components of all living organisms and play critical roles in cellular processes such as energy storage, signaling, and membrane structure. Cholesterol plays a crucial role in animal cells by regulating membrane fluidity as well as the synthesis of hormones, such as estrogen, testosterone, and cortisol. Prenols are derived from the mevalonic acid pathway, which is responsible for the biosynthesis of a wide range of biomolecules, including coenzymes and vitamins. Dysregulation of lipid synthesis has an impact on health, therefore, the study of lipids like sterols and prenols can lead to a better understanding of the role of these lipids in the pathogenesis of diseases.

In lipidomics, mass spectrometry (MS) coupled with chromatography allows for high-resolution separation and sensitive detection. The combination of reversed-phase (RP) chromatography and selected reaction monitoring (SRM) transitions on QqQ instrument provides the separation of isomers and high sensitivity. Some lipid classes are not appropriate for this approach because they do not produce selective fragments. However, such a problem can be solved by the derivatization approach.

The novel derivatization method using 3-(chlorosulfonyl)benzoic acid, which had never been used before for the derivatization of lipids, was developed for the determination of sterols, prenols, and neutral lipids. The reaction enables the detection of these lipid classes in the negative - ion mode, which minimizes the in-source fragmentation common in the positive - ion mode, leading to higher sensitivity. The RP-UHPLC/MS/MS method was optimized based on 30 derivatized standards from 4 lipid classes (sterols, prenols, monoacylglycerols, and diacylglycerols). The derivatization reaction was thoroughly optimized using spiked pooled plasma (9 internal standards from 4 lipid classes), and the molar ratio 6:1 of pyridine with derivatization agent reacting for 1 hour at 60 °C provided the best yield. The Folch extraction was used to eliminate the excess pyridine and derivatization agent. Repeatability and reproducibility were investigated by one operator, respectively, two operators, reporting RSD lower than 15 %. Short-term stability in the autosampler (4 °C) for 18 hours and long-term stability in the freezer at -80 °C for one week at least was determined with RSD less than 10 %. The fully optimized method was applied for the identification of sterols, prenols, monoacylglycerols, and diacylglycerols in human plasma.

n-vivo lipidomics: Towards molecularly-guided autonomous surgical robots

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T21

Histological specificity of structural lipid composition of tissues was originally recognised in the 1970's using TLC and ^{31}P NMR spectroscopy. While it qualified as an interesting observation, molecular spectroscopy-driven histology only became a tangible reality by the late 1990's with the development of mass spectrometric lipid imaging technologies. Since then numerous publications using SIMS, MALDI and DESI-MSI have reported cell type-specific structural lipid signatures, predominantly based on the distribution of glycerophospholipids and sphingolipids observed. Rapid evaporative ionisation mass spectrometry was introduced 15 years ago to take this histological application one step further and offer a solution for the long-standing problem of intrasurgical tissue identification. While the REIMS technique uses various surgical energy devices as ambient ion sources, the resulting spectral information is always dominated by structural lipid signals.

Surgical microrobotics utilising laser dissection has been demonstrated to work with cellular precision. The main limitation for these devices is the rate of information processing by the human operator. If a surgeon is using a microscope and working at cellular precision facilitated by the microrobot, the resection of an average-sized brain tumour would take several days of continuous work, which is obviously not feasible. An obvious solution for this problem is to close the decision-making loop and construct an autonomous surgical robot, which uses the in-situ lipidomic information for identifying cancer cells on the surgical area. While the approach still involves the ablation of healthy cells, the amount of healthy tissue damaged is 2-3 orders of magnitude lower, compared to traditional surgical approaches using set margin.

The Molecularly Aware Robotics Surgery (MARS) platform consists of four core elements: the Rapid Evaporative Ionisation Mass Spectrometry (REIMS) system responsible for the tissue ablation (using a CO2 surgical laser) and molecular characterisation. The optical/computer vision system comprised of stereo cameras for 3D reconstruction of the target area and accurate position estimation of the robot position The inflatable soft robot consists of three inflatable pouches that can be deployed while deflated, and provide a scaffolding and pneumatic actuation when inflated. And finally, the electrothermal fibre robot provides precision motion through thermal actuation and facilitates the delivery of the laser energy into the ablation area and the removal of the sample aerosol after the ablation through integrated channels inside the fibre. The optical imaging system provides the initial information on the location of the surgical resection target, and it tracks the 3D position of the robotic platforms during movement. The MS platform provides biochemical information during ablation that can classify the ablated materials' histological status. Based on the information provided by the sensing, the robotic platform can be positioned using the flexible hydraulic soft robot platform and using the fibre robot, and precision ablation can be performed. The successful coupling of all components enables the system to be used in a user-independent mode, where the decision of the ablation motion will be based on the signal and feedback provided by the mass spectrometer through performing real-time molecular diagnostics. The system has been successfully tested on mouse models of basal cell carcinoma ex-vivo and human testing is under way.

Unveiling the Secrets of Lipid Transport In Vivo using METALIC

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T22

The distinct activities of organelles depend on the proper function of their membranes. Coordinated membrane biogenesis of different organelles necessitates lipid transport from their site of synthesis to their destination. Several factors have been proposed to participate in lipid distribution, but despite its basic importance, in vivo evidence linking the absence of putative transport pathways to specific transport defects remains scarce. A reason for this scarcity is the near absence of in vivo lipid trafficking assays. Here we introduce a versatile method named METALIC (Mass tagging-Enabled TrAcking of Lipids In Cells) to track interorganelle lipid flux inside cells. In this strategy, two enzymes, one directed to a 'donor' and the other to an 'acceptor' organelle, add two distinct mass tags to lipids. Mass-spectrometry-based detection of lipids bearing the two mass tags is then used to quantify exchange between the two organelles. By applying this approach, we show that the ERMES and Vps13–Mcp1 complexes have transport activity in vivo, and unravel their relative contributions to endoplasmic reticulum–mitochondria lipid exchange.

Alkyne lipid tracers and the role of MFSD2a for lipid import into the brain

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T23 **PRIZE**

Phospholipids with a choline head group are an abundant component of cellular membranes and are involved in many important biological functions. Major facilitator superfamily domain-containing protein 2 (MFSD2a) is a membrane protein which is expressed at the blood-brain barrier (BBB), specifically in endothelial cells. Amongst other functions MFSD2a was attributed a role in docosahexanoic acid (DHA) transport into the brain. Esterified to lysophosphatidylcholines (LPCs) the DHA is imported by an unclear mechanism. For studies on this process a set of tracer LPCs where propargylcholine replaces the choline head group or an alkyne fatty acid (FA) serves as side chain are presented here. These labeled precursors direct the traceable tag with superb specificity and efficiency to the primary target lipid class. Also, a novel strategy to analyze propargylcholine phospholipids by mass spectrometry is described. Using azido-palmitate or azido-C171 as click-reporters for both propargylcholine phospholipids and other lipid metabolites a highly specific, sensitive and robust method is introduced. A first study applying the new technique for investigation of the local choline phospholipid metabolism and the lipid transport at the BBB as well as the role of Mfsd2a for this process is presented.

Motor Neuron Disease or Sensory Neuropathy? L-Serine as a modulating factor

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T24

Introduction:

Hereditary Sensory Neuropathy Type 1 (HSAN1) is a progressive sensory neuropathy caused by mutations in the enzyme Serine-palmitoyltransferase (SPT). SPT catalyzes the rate-limiting step in the de-novo synthesis of sphingolipids. HSAN1 mutations induce a permanent change in the substrate specificity of SPT shifting from the canonical substrate L-Serine to the alternative L-Alanine. This forms an atypical class of neurotoxic 1-deoxySphingolipids.

In contrast, amyotrophic lateral sclerosis (ALS) is a progressive, neurodegenerative disease affecting lower and upper motor neurons. Clinical hallmarks include progressive muscle atrophy, speech and swallowing difficulties, fasciculation, altered reflexes, and spasticity. Recently, we reported five heterozygous variants in SPT identified in eight unrelated families with childhood ALS.

Methods:

We analyzed the sphingolipid profile in blood of affected individuals and cellular models expressing the SPT-ALS variants by high-resolution mass spectrometry. We used stable isotope labelling to characterize activity of the mutants and their impact on sphingolipid de-novo synthesis.

Results:

Different from SPT variants that cause HSAN1, the dominantly acting SPT-ALS variants cluster in exon 2. This domain is important for the interaction with the regulatory SPT subunit ORMDL3. Consequently, all SPT-ALS variants showed a reduced homeostatic control causing an excessive de-novo formation of ceramides and other SL species. Restricting the SPT substrate L-Serine, reduced the formation of canonical SL but caused an increased formation of 1-deoxySL instead. This indicated that low L-serine might cause a phenotypic shift from a motor to a sensory phenotype. This was confirmed in an SPT-ALS family which members showed either a sensory or a motor phenotype despite having the identical variant.

Conclusions:

Mutations in SPT can either cause the sensory neuropathy HSAN1 or result in motor neuron degeneration and childhood ALS. Limiting L-serine availability causes a metabolic and phenotypic shift from the motor to the sensory phenotype.

Accurate lipid quantification without fragmentation bias

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T25

Lipid quantification on the molecular species level is based on the abundance of specific fragments detected in MSn spectra of endogenous lipids and standards. As species-specific internal standards are commonly not feasible, lipids are quantified utilizing only one or few internal standard(s) per class. However, the analytical response of individual lipid species is determined by its lipid class and other structural elements like double bonds, hydroxy groups, and chain length. This tutorial covers developing and applying fragmentation models for sphingolipids and glycerophospholipids, aiming to overcome challenges in accurately quantifying these critical biomolecules. The determined response factors are based on experimental data and are independent of the employed instrumentation, collision energies, and internal standard(s).

Omics-Scale High-Throughput Targeted LC-MS/MS Approach for Lipid Profiling: From Development to Application

Medina, Jessica[1], Borreggine, Rebecca[1], Teav, Tony[1], Gao, Liang[2,3], Ji, Shanshan[3], Carrard, Justin[4], Jones, Christina[5], Blomberg, Niek[6], Schmidt-Trucksass, Arno[4], Giera, Martin[6], Cazenave-Gassiot, Amaury[2,3], Gallart-Ayala, Hector[1], **Ivanisevic, Julijana**[1]

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T26

Detailed lipid analysis represents a valuable opportunity for clinical applications due to the essential roles that lipids play in metabolic health. However, a comprehensive and high-throughput lipid profiling remains challenging given the lipid structural complexity and exceptional diversity. Herein, I will present an 'omic-scale targeted LC-MS/MS approach for the straightforward and high-throughput quantification of a broad panel of complex lipid species across 26 lipid (sub)classes. The workflow involves an automated single-step extraction with 2-propanol, followed by lipid analysis using Hydrophilic Interaction Liquid Chromatography (HILIC) in a dual-column setup coupled to tandem mass spectrometry with data acquisition in timed-selective reaction monitoring (t-SRM) mode (12 min total run time). The analysis pipeline consists of an initial screen of 2100 lipid species, followed by high-throughput quantification of robustly detected species. Lipid quantification is achieved by a single-point calibration with 75 isotopically labeled standards representative of different lipid classes, covering lipid species with diverse acyl/alkyl chain lengths and unsaturation degrees. When applied to human plasma, 795 lipid species were measured with median intra- and inter-day precision of 8.5 % and 10.9 %, respectively, evaluated within a single and across multiple batches. The concentration ranges measured in NIST plasma were in accordance with the consensus intervals determined in previous ring-trials. To benchmark our workflow, we characterized NIST plasma materials with different clinical and ethnic backgrounds and analyzed a sub-set of sera (n=81) from a clinically healthy elderly population. Our quantitative lipidomic platform revealed the sex-specificity of the serum lipidome, highlighting numerous statistically significant sex differences. In addition to circulatory lipid profiling for improved clinical prognosis and diagnosis, this approach can also be applied to gain further mechanistic insights through tissue profiling. As organismal lipidome is remarkably diverse and its composition and abundance are determined by tissue-specific *de novo* synthesis and metabolization of external lipid sources, we have also evaluated the relative distribution and diversity of lipid species across four organs playing a central role in the lipid energy metabolism (including lipid synthesis, utilization, and storage): subcutaneous white adipose tissue (scWAT), brown adipose tissue (BAT), skeletal muscle (i.e., quadriceps) and liver.

Profiling and quantitation of bile acids in human biofluids by LC-TIMS-MS

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T27

Bile acids (BAs)and their conjugates are important players in central physiological processes related to human health [1]. Their analysis by LC-MS/MS, however, is often hampered by the occurrence of isobaric and isomeric species differing only in the position or orientation of hydroxyl or carbonyl groups on the steroid scaffold. Co-elution of BAs and derivatives with similar or identical accurate masses and fragmentation behavior necessitates increased selectivity in MS-based workflows for these compounds.

Here we illustrate the utilization of trapped ion mobility spectrometry (TIMS) to separate and annotate co-eluting isobars in an optimized LC-TIMS-MS method. The method, utilizing the timsTOF Pro 2 system coupled to an Elute UHPLC system via VIP-HESI source (Bruker, Bremen, Germany) provided quantitation and profiling capabilities for BAs extracted from human biofluids. TIMS can measure BAs specific Collisional Cross Section (CCS). These CCS values are an effective tool for ensuring data reproducibility within and across laboratories where bile acid LC retention times may differ, limiting confident annotation and replication.

Mixtures of pure reference standards (Cambridge Isotope Labs) were analyzed using the optimized LC-TIMS-MS method. Applying a C8-reversed phase (RP) method (Sarafian *et al.*, 2015 [2]), 71 BAs could be separated and annotated in the standard mixture using TASQ 2023 and MetaboScape 2023 software packages for targeted quantitation and non-targeted profiling, respectively. Co-eluting isomers such as lithocholic acid and allolithocholic acid could be mobility separated as [M+acetate]$^-$ ions.

Furthermore, inter-technology (TIMS vs. drift-tube, [3] and inter-laboratory assessments of CCS values using BA mix standards showed an average CCS value deviation of 0.1 % (n=6) and 0.3 % (based on 2 labs, 8 compounds), respectively.

In a qualitative profiling experiment based on the target list of 71 BAs, 25 bile acids were annotated in the SRM 1950 serum extract (NIST Standard Reference Material 1950 human reference plasma). Annotation quality (AQ) was assessed based on AQ score, a visual tool in MetaboScape incorporating multiple molecular identifiers (e.g., CCS and m/z). Data visualization in Kendrick mass defect plots facilitated annotation of isoglycocholic acid, which had not been included in the original target list.

The quantitative capabilities of the established assay were estimated based on the linear dynamic range and limit of detection (LOD) for selected BAs. Taurocholic acid was measured with a linear dynamic range of 3.7 orders of magnitude and an LOD of 300 pM. For the BAs that were detected within the dynamic range of the LC-TIMS-MS method all determined concentrations in SRM1950 extracts were within the range of the published standard uncertainty locations [4].

[1] DOI:10.1038/s41586-020-2047-9
[2] DOI:10.1021/acs.analchem.5b01556
[3] https://mcleanresearchgroup.shinyapps.io/CCS-Compendium
[4] DOI:10.1194/jlr.M079012

Recent methodological advances of MALDI-2 and t-MALDI-2 mass spectrometry for molecular imaging of lipids

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T28

MALDI mass spectrometry combined with laser-postionization (MALD-2) is relatively new technology that enables a significant ion boost for several classes of lipids [1]. Due to the low amounts of sample available per pixel these improvement is of particularly relevance in high-resolution MALDI imaging applications. To achieve a spatial resolution in the 1 µm-range, transmission mode (t-)MALDI-2 can furthermore be applied [2,3]. In this geometry, samples are irradiated from the "back side", a feature enabling the use of microscopy-grade objectives. At the same time, light collected from the sample via this objective enables obtaining bright-field as well as fluorescence images directly from the sample as mounted in the ion source.

In my talk, I will highlight the potential of the MSI and multimodal approaches for the analysis of sterols, phospho- and glycolipids, and bacterial lipopeptides. Selected application examples include murine and invertebrate tissues, microbial biofilms, and eukaryotic cell cultures.

[1] Soltwisch J, Kettling H, Vens-Cappell S, Wiegelmann M, Müthing J, Dreisewerd K. Mass spectrometry imaging with laser-induced postionization. Science 2015, 348, 211-215.

[2] Niehaus M, Soltwisch J, Belov ME, Dreisewerd K. Transmission-mode MALDI-2 mass spectrometry imaging of cells and tissues at subcellular resolution. Nat. Methods 2019, 16, 925-931.

[3] Bien T, Koerfer K, Schwenzfeier J, Dreisewerd K, Soltwisch J. Mass spectrometry imaging to explore molecular heterogeneity in cell culture. Proc. Natl. Acad. Sci. U.S.A. 2022, 119, e2114365119.

Optimisation and Application of Single Cell Lipidomics

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T29

The metabolic content of single cells has been a rapidly progressing area of research in the last decade of metabolomics. Single cell heterogeneity has been demonstrated in monoclonal cell populations due to differing epigenetic environments. Therefore, analysis at the single cell level is able to yield observations which are masked by bulk population analysis in myriad studies of cell populations, including infectious disease, immunology and ageing.

Single cell lipidomics methods using mass spectrometry are still in the early stages of their development. Therefore, exploring changes to current methodology could yield significant improvements in the coverage of metabolites obtained from a single cell. Nanocapillary sampling can be used to sample whole living cells from 2D culture using a microscope-guided nanocapillary. This approach not only allows the sampling of living cells, but also retains spatial information. Nanocapillary sampling could therefore be used to investigate cell-cell interactions and location-specific effects.

Our group have recently coupled this approach to liquid chromatography mass spectrometry (LC-MS) to measure the lipidomic profile of single cells. Once cells are collected, lysing solvent can be backfilled into the capillary before transfer to a vial for analysis by LC-MS. In this work we describe the optimisation of this approach i.e. improving sensitivity and the coverage of lipid classes as well as the incorporation of MS/MS to putatively identify lipids. We have recently used this approach to determine the impact of chemotherapy treatment on live single cells, finding significant changes in the lipidomic profile.

Furthermore, the University of Surrey has recently been awarded funding from the BBSRC to host a UK National Facility for spatially resolved single cell "omics" (SEISMIC), using a newly commercialised platform for nanocapillary sampling. This will be an open resource, with grants for researchers to visit to conduct experiments at our site. Plans for delivery of this facility, and opportunities for collaboration will be presented.

Hidden obstacles in clinical lipidomics –Ex vivo stabilities of lipid species in blood

Wang, Qingqing[1], Liu, Xinyu[1], Liebisch, Gerhard[2], Ahrends, Robert[3], Ekroos, Kim[4], Hu, Chunxiu[1], Birkenfeld, Andreas[5], Peter, Andreas[6], Xu, Guowang[1], **Lehmann, Rainer**[6]

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T30

Clinical lipidomics is attracting interest from more and more scientists. This goes along with increases in the collection of blood samples for this purpose and use of already collected biobank samples. However, the situations for blood collection and processing differ between individual hospitals and study wards. Furthermore, lipid species show quite different *ex vivo* stabilities in whole blood. Consequently, a risk for misinterpretations, irreproducibilities or discrepancies of clinical lipidomics data exist. Increases in standardization and harmonization of lipidomics approaches are currently under debate to avoid such situations (1, 2).

As part of the "Preanalytics interest group" of the International Lipidomics Society (ILS) we studied in EDTA-whole blood the stability of >400 lipid species representing 13 lipid classes. Covering common daily routine conditions in clinical settings for blood processing and transportation whole blood was exposed to either 4°C, 21°C, or 30°C at six different time points (0.5h to 24h) directly after drawing of blood. More than 800 samples were analyzed by UHPLC-high-resolution mass spectrometry.

We detected 325 and 288 robust lipid species with no need for cooling or timely processing, meaning they showed no significant change even after 24h exposure of EDTA whole blood to 21°C or 30°C, respectively. To achieve valid data for all covered, more than 400 lipids the best preanalytical sample handling was to cool whole blood at once, permanently, and finish transportation and separation of plasma from blood cells within 4h. Less suitable as potential biomarkers were FA, LPE and LPC, representing the lipid classes with the lowest stability under all studied conditions in our setting.

In conclusion, the data of our preanalytical study may support the efforts of the ILS to standardize and harmonize clinical lipidomics approaches. Most promising lipids for proper future use in a diagnostic pattern or profile should come from the list of robust lipid species, because the blood samples can be treated as all other patient samples in the hospital. But also, for all covered less stable lipids a preanalytical conditions was detected to achieve reliable and comparable data independent from the place where the samples were collected and processed, which is suitable to be fulfilled in clinical settings.

1. Liebisch, G., R. Ahrends, et al. (2019). Lipidomics needs more standardization. *Nat Metab* **1**: 745-747.

2. McDonald, J. G., C. S. Ejsing, et al. (2022). Introducing the Lipidomics Minimal Reporting Checklist. *Nat Metab* **4**: 1086-1088.

Metabolomic Profiling of Plasma Samples from PPMI Identifies Molecular Signatures of Parkinson's Disease and Genetic Parkinson's Disease

Suh, Jung H.[1], Huntwork-Rodriguez, Sarah[1], Crotty, Grace[2], Macicuca, Romeo[1], Macklin, Eric[3], Davis, Sonnet[1], Alkabsh, Jamal[1], Bakshi, Rachit[2], Chen, Xiqun[2], Molsberry, Samantha[4], Ascherio, Alberto[2], Schwarzchild, Michael[2]

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T31

Objective:
To validate and explore metabolomics-based plasma biomarkers of Parkinson's disease (PD) in *LRRK2* and *GBA* carriers as compared to sporadic PD patients among PPMI participants.

Background:
Prior plasma metabolomic analysis in PD(+) and PD(-) subjects including *LRRK2* and *GBA* mutation carriers replicated significant differences in caffeine-related metabolites in PD(+) versus PD(-) and showed reduced GCase activity in GBA carrier plasma versus non-carriers (Crotty MDS Madrid 2022). Here, we report additional exploratory analysis of metabolomic profiles associated with disease and genetic status.

Design/Methods:
Plasma from 629 PPMI participants selected based on PD (+/-) and genetic status (a *GBA* or *LRRK2* mutation or neither) were analyzed by liquid chromatography coupled to mass spectrometry. 298 plasma analytes met reporting criteria. Normalized analyte levels were compared between groups using robust ANCOVA models for log2 analyte level as the dependent variable and age, sex, PD status, genetic status, levodopa use, and their interactions as independent variables.

Results:
Results replicated predicted PD associations with ergothioneine and caffeine metabolites and *GBA* associations with glucosylsphingosine (GlcSph). *LRRK2* status was uniquely associated with increases in circulating levels of docosahexanoic acid (DHA) and other lipid classes containing DHA including lysophosphatidylcholine (LPC) and bis(monoacylglycero)phosphate (BMP). Additionally, modest but highly specific association between decreased 4-trimethylaminobutanal (TMABA; protein lysine-derived catabolite) and *GBA* status.

Conclusions:
Metabolic profiles show highly distinct associations with PD, *LRRK2* and *GBA* status, potentially delineating differences in underlying biology. While lifestyle/xenobiotic profiles are dominant in PD, unique associations between GlcSph and TMABA and *GBA* status implicated anticipated alteration in lysosomal function. Broad alterations in DHA containing lipids, including LPC-DHA required for brain DHA, were observed only in *LRRK2 (+)* subjects*,* independent of disease and L-DOPA status. Dominant *LRRK2*-dependent lipid profiles show novel associations between systemic DHA homeostasis and *LRRK2* polymorphism. Further studies will be needed to clarify potential causal implications of these findings.

Emerging developments in shotgun lipidomics

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T32

Shotgun lipidomics relies on direct infusion of total (unseparated) lipid extracts into a high resolution mass spectrometer. Despite shotgun produces highly convoluted "snapshot" spectra that encompass the full-lipidome composition, we argue that unique features of shotgun methodology outweigh this added analytical complexity. In shotgun the analyte composition does not change during spectra acquisition. Furthermore, ample acquisition time enables analyzing total extracts by high- and ultra-high resolution mass spectrometry and resolve fine structure of major isotopic peaks. Implications of these inherent shotgun features are multifaceted and open new research avenues such as quantitative lipidomics of liquid and solid biopsies with high inter-laboratory concordance or transition from "static" to "dynamic" lipidome profiling by simultaneous lipidome-wide monitoring of lipids turnover.

Abstracts of Posters

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P02	Platelet lipid metabolism in vascular thrombo-inflammation.	Ahrends, Robert
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P04	Quantitative evaluation of the fatty acid composition of phosphoinositides in biological matrices	Grander, Julia
P05 **PRIZE**	Inhibition of DHCR24 activates $LXR\alpha$ to ameliorate hepatic steatosis and inflammation	Miao, Jing
P06	BACE2 regulates lipid metabolism in cancer	Matafora, Vittoria
P07 **PRIZE**	Changes of lipidomic profile from the bleomycin mice models of pulmonary fibrosis	Kim, Kyeong-Seog
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P17	Introducing the Lipidomics Minimal Reporting Checklist	Kopczynski, Dominik
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P19 **PRIZE**	High-Throughput and Comprehensive Lipidomic Analysis of Human Plasma Using UHPSFC/MS	Peroutkova, Petra
P20 **PRIZE**	LC-MS investigation of artificially oxidized phosphatidylethanolamines in Saccharomyces cerevisiae based on two-dimensional heart-cut liquid chromatography	Wieland, Dominik
P21	New method to characterize epidermal Ceramides by Supercritical Fluid Chromatography (SFC) coupled to High Resolution Mass (Q-Tof): application to various samples with skin barrier default.	Bertrand-Michel, Justine
P22	Description of FAHFAs in Caenorhabditis elegans	Jeck, Viola
P23	Profiling of lipids in human plasma with Total Correlation–Mass Spectrometry (TOC-MS)	Liashenko, Svitlana
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P28	The significance of de novo lipogenesis in platelets	Pirotton, Laurence
P29 **PRIZE**	Mass spectrometry for monitoring and quality control of differentiation of pluripotent stem cells to lung progenitors using biostatistical models	Pečinka, Lukáš
P30	Quantitative Analysis and Lipidomic Profiling of Human Serum of Pancreatic Cancer Patients Focused on Less Abundant Lipid Classes by HILIC-UHPLC/MS	Peterka, Ondřej
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P32	Novel omic Mass Spectrometry (MS) data analysis software applied untargeted LC-MS/MS lipidomics use	Hayashi, Akio
P33	Ether lipid modulation in a preclinical model of dilated cardiomyopathy remodels the cardiac and circulating lipidome and attenuates pathological cardiac morphology	Tham*, Yow-Keat
P34	Analysis of esterified oxylipins in the membranes (oxidized phospholipids) by means of targeted and untargeted LC-MS/MS	Carpanedo, Laura
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P43	Understanding the role of plasmalogen lipid in synaptic function: a cellular neurolipidomics study	Angelini, Roberto
P44 **PRIZE**	Discerning the functional role of plasmalogen at the synapse by shotgun lipidomics	Gane, Iwan
P45 **PRIZE**	Hepatic lipid profiling - Deciphering hepatic lipid metabolism with time and species resolution	Sajeevan, Theja
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P50	Metachromatic leukodystrophy diagnosis in Morocco: exploration of sulfatides diversity in urine samples of infantile patients using molecular networks	FDIL, Naima
P51	Novel lipid biomarkers for algal resistance to viral infection in the ocean	Schleyer, Guy
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P53 **PRIZE**	Data Independent Acquisition for the Dissection of the Unfolded Protein Response in Glioma	Wenger, Alexander
P54	Multidimensional lipidomic reveals specific adipose-derived extracellular vesicle lipid sorting informative of the obesity metabolic state	Lhomme, Marie
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P57	Changes in the mitochondrial phospholipidome of OPA1 and mitofusins knockouts of mouse embryonic fibroblasts: an investigation by hydrophilic interaction liquid chromatography with high-resolution mass spectrometry	Castellaneta, Andrea
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P65	Untargeted hair lipidomics: Comprehensive evaluation of the hair-specific lipid signature and considerations for retrospective analysis	van de Lavoir, Maria
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P72	Ultraviolet photodissociation (UVPD) mass spectrometry for structural characterization of lipids in biological matrices on chromatographic time scales	Ravi Deshpande, Rahul
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Spatial Mapping of Lipids and Elements by Mass Microscopy and Integration with LA-ICP-MS in the Diabetic Mouse Pancreata

Matsuo, Ei-ichi[1], Okuda, Koji[1], Dhal, Jeffrey[2], Matsubara, Toshiya[2], Yamaguchi, Shinichi[1], Gamayot, Jannine[3], Chen, Shiuhwei[3], Kusminski, Christine[3], Scherer, Philipp E.[3], **Gordillo, Ruth**[3]

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P01

Pancreas endocrine function critically relies on the islets of Langerhans that secrete insulin and glucagon as well as other hormones. Glucose homeostasis mainly relies on the opposite glucose lowering and glucose increasing properties of insulin and glucagon. In type-2 diabetes, hyperglycemia is largely the consequence of insufficient insulin secretion and excessive glucagon secretion in the context of insulin resistance. Previous pancreas LC-MS/MS lipidomics studies required islets isolation by a lengthy process involving tissue digestion and manual selection, compromising metabolite basal levels. MALDI-TOF imaging allows for *in situ* global assessment of lipids in pancreata cryosections. Our methodology was applied to a mouse model of diabetes, the adipocyte-specific-doxycycline-inducible mitochondrial ferritin (FtMT) overexpression, which displays massive beta-cell hyperplasia as its most striking phenotype.

Analysis of frozen mouse pancreas cryosections revealed sphingomyelin (SM) 34:1 accumulation in the islets of Langerhans. Results from positive mode analysis display a different distribution of phosphatidyl choline (PC) (34:1) ion clusters $[M-H+K+Na]^+$ and $[M+Na]^+$ in blood vessels which it is attributed to the different microenvironment within the tissue. PC (32:2) $[M+H]^+$ accumulates in blood vessels as well. Negative mode analysis indicated elevated concentrations of alkylacyl phosphatidyl ethanolamine (PE-O) (38:5) $[M-H]^-$ and phosphatidyl serine (PS) (38:4) $[M-H]^-$ in islets while phosphatidyl inositol (PI) (36:4) $[M-H]^-$ is found at lower concentrations. Sulfatide (SHexCer) (34:1) $[M-H]^-$ is also elevated in endocrine pancreata. It has been reported that certain sulfatide species are required for insulin secretion. Molecular images of lipid species overexpressed in islets of Langerhans display beta-cell hyperplasia in the case of the FtMT overexpressing mice. Immuno-fluorescence of washed sample slides posts MALDI-TOF analysis with anti-insulin and anti-glucagon antibodies confirmed overlay with lipids molecular images. Moreover, laser ablation inductively coupled mass spectrometry (LA-ICP-MS) can be combined with MALDI-TOF imaging data providing the spatial distribution of elements on the tissue cryosection. Our preliminary results at 50 μm showed a clear accumulation of Zn in islets. It is known that Zn forms a strong complex with insulin. Fe marks the localization of blood vessels while P is ubiquitous. The analysis of pancreas cryosections at 25 μm allows for differential analysis providing relative abundance of lipid species between pancreatic exocrine and endocrine regions as well as between groups of study. Our preliminary results display striking differences amongst lipid species concentrations between islets and exocrine regions of interest.

Instrumentation. Automated matrix deposition system was achieved with an iMLayer (Shimadzu Corporation, Japan). Imaging mass spectrometry analysis was performed on a IMScope QT mass microscope (Shimadzu Corporation). Spatial elemental analysis was performed on an imageBIO266 (Elemental Scientific Lasers, USA) coupled to an ICPMS-2030 (Shimadzu Corporation). Imaging data processing was performed using IMAGEREVEAL MS software package (Shimadzu Corporation).

Platelet lipid metabolism in vascular thrombo-inflammation.

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P02

The function of platelets - and thereby the balance between thrombosis and hemostasis - critically depends on their lipid composition. At the same time, platelets are capable of interacting with inflammatory cells by releasing lipids in a paracrine manner. Over the years, many studies have emphasized the importance of both, membrane and signaling lipids, in the signaling pathways underlying arterial thrombosis and chronic inflammation. In line with this, an imbalance of platelet lipid homeostasis is associated with thrombo-inflammatory diseases such as acute coronary syndrome. By establishing quantitative platelet lipidomic analysis, an opportunity has arisen to deepen our knowledge about platelet lipids regulating thrombo-inflammation and vice versa. Past and future investigations in this upcoming field are of great clinical importance since they will presumably pave the way for the identification of novel biomarkers. In addition, targeting specific regulators of the platelet lipid metabolism is a promising strategy to receive both anti-thrombotic and anti-inflammatory therapeutics and could be beneficial to a wide variety of patients with vascular thrombo-inflammatory diseases. This review summarizes the latest scientific findings in the field of platelet lipidomics research and does so by focusing on the metabolism of sphingolipids, oxylipins and phosphoinositides, which are affected by dynamic modifications in a pathophysiological manner. Further, this review elucidates the impact of these platelet lipids on thrombo-inflammatory cardiovascular diseases and highlights potential diagnostic and therapeutic targets.

Septic shock is associated with significant changes in the platelet lipidome

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P03 PRIZE

Background. According to the WHO, sepsis is responsible for one in five deaths worldwide. The syndrome is defined as life-threatening organ dysfunction caused by an impaired host immune response to infection. Sepsis is characterised by major endothelial dysfunction, microvascular alterations, and coagulopathy. In addition to their involvement in pathological haemostatic processes, platelets are key players in sepsis as they promote immunothrombosis, notably by generating cytokines and lipid mediators of inflammation. It is increasingly recognised that the composition of the platelet lipidome is critical to their function. Sepsis is associated with major perturbations in cell signalling and metabolism, the impact of which on lipid metabolism has already been demonstrated in various cells. However, the lipidomic profile of platelets during sepsis has never been studied.

Aim. The aim of this study is to investigate the platelet lipidome of septic patients. The additional objective of this analysis is to explore the potential association between altered lipids and platelet reactivity, as well as to identify new potential biomarkers of sepsis.

Method. Platelets were isolated from 48 septic and 48 control patients with similar age, sex, and comorbidities. Lipidomic analysis was carried out by untargeted liquid chromatography–mass spectrometry (QTOF). Clinical parameters of the patients were measured by enzyme-linked immunoassay (ELISA).

Results. Lipidomic analysis identified 225 species and showed significant changes in the lipid composition of platelets during sepsis. Platelets from patients with septic shock showed increased levels of diacyl and triacylglycerols, as well as ceramides and deoxyceramides. A concordant decrease in sphingomyelin species was also observed. Excessive ceramide formation has been associated with multiple disorders, including atherosclerosis and cardiovascular disease. Regarding phospholipids, patients showed a reduction in lysophospholipids as well as alterations in the composition of fatty acid chains. An increase in short and (un)saturated fatty acid chains was observed and associated with a substantial reduction in phosphatidylcholines and phosphatidylethanolamines containing long polyunsaturated fatty acid chains (ω3 and ω6). The latter are key phospholipids for the generation of pro- and anti-inflammatory lipid mediators.

Conclusion. Our data reveal that critical changes in the platelet lipidome occur during sepsis. Upregulated lipids are mainly glycerolipids and ceramides, while lysophospholipids are drastically reduced. These changes as well as alterations in the composition of the fatty acyl chains of phospholipids might play a role in the pathophysiology of the disease.

Quantitative evaluation of the fatty acid composition of phosphoinositides in biological matrices

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P04

Cytotoxic stress has severe consequences on the fatty acid composition of cellular phospholipids and impacts cell fate decisions. We recently found that mechanistically diverse stressors lower the ratio of monounsaturated (MUFAs) to saturated (SFAs) and polyunsaturated fatty acids (PUFAs) in membrane phospholipids, specifically in phosphatidylinositols (PI) (Thürmer *et al.*, 2022). This metabolic reprogramming is caused by a drop in the expression of stearoyl-CoA desaturase (SCD-1), an enzyme that incorporates a Δ9-*cis*-double bond into SFA-coenzyme A. Phosphoinositols are converted into phosphoinositides (PtInsP), a set of low abundant phospholipids that orchestrate a variety of biological processes. Whether phosphoinositides with defined fatty acid composition exhibit different biological functions is poorly understood, as is their regulation by internal and external stress factors. To obtain insights into these processes, we established a targeted UPLC-ESI-MS/MS method that allows us to discriminate between phosphoinositides based on headgroup and fatty acid composition. Starting from a previously published method (Clark *et al.*, 2011), our workflow involves i) the methylation of inositol-bound phosphates, ii) liquid/liquid extraction under acidic conditions, iii) separation of PtInsP on a polyphenyl column, and iv) detection of both headgroup and acyl fragments by multiple reaction monitoring in the positive ion mode, followed by v) normalization of the signals to headgroup-specific internal standards. As proof-of-concept, we analyzed the fatty acid composition of $PtInsP_2$- and PtInsP-species in triple-negative human MDA-MB-231 breast cancer cells and detected 15 $PtdInsP_1$ and 23 $PtdInsP_2$, with 18:0_20:4-, 18:0_20:3- and 18:0_18:1- species being most abundant, which is in line with previous reports (Barneda *et al.*, 2022). In conclusion, we established a sensitive method to quantitatively determine changes in the phosphoinositide fatty acid composition under pathophysiological stress conditions.

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Inhibition of DHCR24 activates LXRα to ameliorate hepatic steatosis and inflammation

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P05 **PRIZE**

Liver X receptor (LXR) agonism has theoretical potential for treating NAFLD/NASH but synthetic agonists induce hyperlipidemia in preclinical models. Desmosterol, which is converted by Δ24-dehydrocholesterol reductase (DHCR24) into cholesterol, is a potent endogenous LXR agonist with anti-inflammatory properties. We aimed to investigate the effects of DHCR24 inhibition on NAFLD/NASH development. Here, by using APOE*3-Leiden.CETP mice, a well-established translational model that develops diet-induced human like NAFLD/NASH characteristics, we reported that SH42, a selective DHCR24 inhibitor,
markedly increases desmosterol levels in liver and plasma, reduces hepatic lipid content and the steatosis score, and decreases plasma fatty acid and cholesteryl ester concentrations. Flow cytometry showed that SH42 decreases liver inflammation by preventing Kupffer cell activation and monocyte infiltration. LXRα-deficiency completely abolishes these beneficial effects of SH42. Together, inhibition of DHCR24 by SH42 prevents diet-induced hepatic steatosis and inflammation in a strictly LXRα–dependent manner without causing hyperlipidemia. Finally, we also showed that SH42 treatment delays NAFLD progression from simple steatosis to NASH. In conclusion, we anticipate that pharmacological DHCR24 inhibition may represent a novel therapeutic strategy for treatment of NAFLD/NASH.

BACE2 regulates lipid metabolism in cancer

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P06

Lipid metabolism represents one of the most deregulated pathways in cancer. Enhanced lipid synthesis, storage, and uptake contribute to cancer proliferation, survival, invasion and metastasis[1]. Lipids serve to obtain energy, constitute biological membranes, and act as signaling molecules. Rewiring of lipid metabolism in cancer has been linked to the activation of oncogenic signaling pathways as well as to the cross talk with tumor microenvironment[2]; however, the mechanism adopted by cancer cell to became addicted to lipids is still unknown. By comparative analysis of proteins secreted by metastatic and primitive melanoma cell lines, our group recently found that proteins involved in lipid metabolism such as ApoE, together with amyloidogenic proteins, accumulate markedly in the metastatic extracellular environment[3]. We also observed that BACE2 sheddase activity is responsible for amyloid maturation, and that its inhibition increases the secretion of proteins related to lipid metabolism in metastatic cells. From global lipidomic profiles of melanoma cell lines using an in-house optimized LC-MS method, Opti-nQL [4], we found that metastatic cells, which express higher levels of BACE2 compared to the primitive ones, are enriched in free cholesterol and triglycerides and accumulate lipid droplets. By proteomic analysis, we also found an increased expression of enzymes related to cholesterol biosynthesis and fatty acids regulation. Of note, upon BACE2 inhibition, lipidomic profiles of melanoma metastatic cell lines showed an increase of free cholesterol and phospholipids and a decrease in triglycerides. Coherently, a decrease in lipid droplets content was observed, suggesting the involvement of BACE2 activity in fatty acid homeostasis. As BACE2 is a sheddase, we focus on its specific role and, by spatial proteomic, we discovered that BACE2 regulates the level of lipids transporters on the membrane, tuning lipid uptake from the extracellular space. We found the same mechanism also present in pancreatic cancer cells displaying high BACE2 level. Our study reveals the role of BACE2 as modulator of lipid metabolism in cancer.

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Changes of lipidomic profile from the bleomycin mice models of pulmonary fibrosis

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P07 **PRIZE**

Idiopathic pulmonary fibrosis (IPF) is a chronic, progressive, and irreversible lung disease. Alterations in lipids were recognized as potential underlying drivers of IPF. To comprehensively understand the lipid profiles in IPF, untargeted lipidomics were performed from 14 bleomycin (BLM) induced mice models and 10 healthy mice models. Intratracheal injection of BLM to a C57BL/6J mice aged 12 weeks to induce pulmonary fibrosis and saline was treated to a healthy mice model. After 14 days of treatment, lung tissue and serum were collected after sacrifice. We have found 154 lipids and 252 lipids from mouse lung tissue and serum, respectively. The separated clustering between BLM and healthy mice were observed in each lung tissue and serum by principal component analysis. Interestingly, the separated profiles were also observed in specific lipid class. Triacylglycerides were depleted in BLM lung tissue, however, surged in BLM serum. Phosphatidylcholines were increased in both BLM tissue and serum. Saturated fatty acids were not changed in both lung tissue and serum, whereas unsaturated fatty acids were surged in BLM lung tissue. The altered lipid profiles obtained from BLM mice models could be a potential target to understanding the underlying mechanism of IPF. To confirm the altered lipid profiles are fibrotic region specific, mass spectrometry imaging analysis will perform in future study.

The feasibility of machine learning approaches in discovering plasma lipid biomarkers for diagnosis of chronic liver diseases

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P08 **PRIZE**

Chronic liver disease can be caused by various factors, including alcohol consumption, virus infections, non-alcoholic fatty liver disease (NAFLD), etc. NAFLD is a condition in which fat accumulates in the liver, leading to inflammation over time. It is usually a benign condition, but in some cases, it can progress to a more severe form called non-alcoholic steatohepatitis (NASH). For diagnosing NAFLD and NASH, liver biopsy is the gold standard but is not indicated in all patients with suspected disease. Recent research has shown that lipidomics can provide valuable insights into the pathogenesis and progression of liver diseases. Therefore, lipidomics can be an ideal tool to identify potential biomarkers of NAFLD and NASH, which can aid in diagnosis, prognosis, and treatment. Here we used the quantified plasma lipid data of chronic liver disease patients, including NAFLD (n=12), NASH (n=12), and alcoholic hepatitis (AH, n=8). However, common statistical methods such as the student t-test or ANOVA have limitations in handling high-dimensional and multicollinear lipidomics data. Recently, machine learning (ML) algorithms caught people's eye for handling high-dimensional datasets with many variables. In this study, both wilcoxon rank sum test and lasso were adopted to select disease-related biomarkers by comparing the different patients. Two different ML approaches were adopted to construct predictive models by the selected lipids. As a result, this study presented the feasibility and robustness of using ML approaches for selecting biomarkers and constructing predictive models in lipidomics analysis.

Critical shifts in lipid metabolism modulate megakaryocyte differentiation and proplatelet formation

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P09 PRIZE

During megakaryopoiesis and consecutive platelet production, megakaryocytes undergo huge cellular morphological changes which are associated with the reprogramming of signaling pathways. Moreover, membrane composition and lipid signaling are expected to be strongly modified. However, the knowledge of how lipids are modulated and which pathways are involved is still lacking.

Here, we use a lipid-centric multiomics approach applying the SIMPLEX protocol [1] to create a quantitative map of the murine megakaryocyte lipidome during maturation and proplatelet formation.

Our data reveal that differentiation is associated with enhanced expression of lipid-related enzymes and driven by an increased fatty acyl import and *de novo* lipid synthesis, resulting in the modulation towards an anionic membrane phenotype. Using inhibitors of fatty acid import and phospholipid synthesis proved to block membrane remodeling and directly reduced megakaryocyte polyploidization and proplatelet formation.

The anionic lipid shift and the upregulation of diacylglycerols were paralleled by the activation of lipid-dependent kinases. Overall, this study provides a framework for understanding how membrane lipid remodeling impacts kinase signaling and proplatelet formation while providing a knowledge base to exploit megakaryopoiesis.

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Yes, it matters: in contrast to blood plasma, serum metabolomics is confounded by platelets

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P10 — PRIZE

Metabolomics is an emerging and powerful molecular profiling method supporting clinical investigations. For clinical metabolomics studies, serum is commonly used. Serum is collected after blood coagulation, a complex biochemical process involving active platelet metabolism. This may proof relevant as platelet counts and function may vary substantially in patients. Applying a multi-omics analysis strategy comprising proteins and metabolites with a focus on lipid mediators, we systematically investigated serum and plasma obtained from the same healthy donors. While Biocrates MxP Quant 500 results correlated well (n=461, R^2=0.991), lipid mediators (n=77, R^2=0.906) and proteins (n=322, R^2=0.860) differed substantially between serum and plasma. Actually, secretome analysis of activated platelets identified all proteins and most lipid mediators significantly enriched in serum when compared to plasma. Furthermore, a prospective, randomized, controlled parallel group metabolomics trial was performed, monitored by serum and plasma analyses. Healthy individuals received either acetylsalicylic acid, affecting platelets, or omega-3 fatty acids, hardly affecting platelets, for a period of seven days. In the acetylsalicylic acid group, serum analysis apparently demonstrated a significant drug-induced downregulation of the lipid mediators TXB2 and 12-HETE. The absence of these observation in plasma analyses suggested that these drug effects took place only during blood coagulation. Other effects of acetylsalicylic acid on alpha-linolenic acid and the fatty acid composition of triglycerides were detected both in serum and plasma. In the omega-3 fatty acid group, serum and plasma analysis results did not differ. These data strongly support the hypothesis that the serum metabolome may be substantially confounded by platelets.

Exploring ether lipid metabolism and obesity in large population cohorts using lipid ratios and GWAS

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P11 — PRIZE

Dysregulated lipid metabolism underlies much of the disease pathogenesis of metabolic disease. Lipidomic profiling of large population cohorts has revealed that reduced levels of plasma ether lipids, a unique class of glycero- and glycerophospho-lipids, are implicated in obesity. Considerable evidence demonstrates the protective capabilities of ether lipids in obesity, including their antioxidant properties and role in regulating thermogenesis. Despite this knowledge, little is known about the complex relationship between ether lipid metabolism and obesity. Here, we utilised lipid ratios, as an alternative measure of biological functions, and genome-wide association studies (GWAS) to define specific pathways of ether lipid metabolism in the setting of obesity.

To achieve this, we analysed plasma lipidomic data from two independent cohorts, including 706 individual lipid species across 36 classes from the Australian Diabetes, Obesity and Lifestyle Study (AusDiab, n=10,339) and 596 individual lipid species across 33 classes from the Busselton Health Study (BHS, n=4,793). We generated 58 ratios, between specific lipid precursor and product pairs, to capture ether lipid composition, enzymatic activity and key pathways involved in ether lipid and fatty acid synthesis. We performed linear regression analysis, adjusting for age and sex, to assess the association between each lipid ratio and various markers of obesity. GWAS was performed on the lipid ratios using imputed genotype (13.8 million SNPs) data from BHS to identify genetic variants associated with changes in ether lipid synthesis.

Body-mass-index (BMI) was inversely associated with total ethanolamine plasmalogen levels relative to total phospholipids (-0.02 SD-change per unit BMI, p-value 1.47×10^{-24}). Ratios capturing key steps in plasmalogen synthesis, such as the addition of the vinyl-ether linkage via phosphatidylethanolamine-N-methyltransferase (PEDS1), had a negative association with BMI (-0.02 SD-change per unit BMI; p-value 2.37×10^{-25}), while ratios detailing catabolic pathways, such as the hydrolysis of ether lipids into lyso-species via calcium-independent phospholipase A2 (iPLA2), demonstrated a positive association (0.09 SD-change per unit BMI; p=2.83×10^{-06}) with BMI. Lipid ratios representing key steps in polyunsaturated fatty acid metabolism showed divergent associations with n-3 and n-6 fatty acyl-chains. Specifically, we observed a strong negative association through the earlier stages of the pathway and a subsequent positive association through the final stage, suggesting peroxisomal dysfunction. These results were consistent across both datasets. GWAS showed a strong association between the alkyl-phosphatidylethanolamine [PE(O)] over alkenyl-phosphatidylethanolamine [PE(P)] ratio and single-nucleotide polymorphisms (SNP) in the PEDS1 gene region (p $<5 \times 10^{-12}$), whilst the PE(P) over alkenyl-phosphatidylcholine [PC(P)] ratio highlighted choline plasmalogen specific associations with SNPs in the TMEM229b region (p$<5 \times 10^{-22}$).

Our analyses illustrate the effects of elevated BMI and obesity on ether lipid metabolism, and demonstrate the potential of population lipidomics and GWAS to define new relationships between obesity and ether lipid metabolism.

Lipids-the key players in cancer cachexia

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P12

Cancer cachexia (CC) is a life-threatening, paraneoplastic syndrome defined as the progressive loss of body weight that cannot be addressed by conventional nutritional support. It is a result of imbalance between energy requirements and energy uptake which further leads to atrophy of skeletal muscles and adipose tissue (AT). So far there is no standardized treatment for CC that can reverse this disorder and this is the reason why we urgently need a new therapeutic approach to tackle CC.

White adipocytes (WAs) store lipids in form of triglycerides, but in a period of starvation, they can also provide energy in form of fatty acids through lipolysis. It is important to emphasize that increased lipolysis in AT is one of the earliest markers of pre-cachectic stage. Lipids play a role in numerous cellular processes including cell growth, differentiation, and signaling. Alterations in lipid metabolism have been implicated in development and progression of cancer. This is the reason why we postulate that unravelling the role of lipids will enable us to better understand the communication between adipocytes and cancer cells during CC.

Our 2D and 3D *in vitro* models showed alterations in lipids in adipocytes as well as in cancer cells. Additionally, analysis of tumors, adipose tissues and serum samples of mice bearing different types of tumors showed changes in lipidomic profile.

These data could be a base for the development of new therapeutic approaches to treat this wasting syndrome.

Optimisation of preanalytical tissue processing workflow for mass spectrometry based quantification of animal and human pancreatic tissue lipidomes

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P13

Many serious disorders, such as fatty liver disease, type 2 diabetes, chronic kidney disease, or various cancer types, are associated with lipid metabolism dysregulation [1]. Consequently, qualitative and qualitative changes in systematic and/or tissue-specific lipidomes can be observed. Therefore, the search for new lipid biomarkers of clinical significance appears to be of great value because of the possibility of their application in the detection of various pathological conditions. Of particular note are some cancer types, including pancreatic ductal adenocarcinoma (PDAC), which does not show specific symptoms in the early stages, making the diagnosis by commonly used screening tests especially difficult [2]. Among the biological samples, biofluids, including plasma, serum, and urine, are commonly used in cancer research [3], while tissue biopsies are more difficult to obtain and tend to be challenging to analyse. On the other hand, tissue lipidomics can provide additional information regarding potential biomarkers and contribute to a deeper understanding of the mechanism of pathology, e.g., when using animal disease models.

Compared to biofluids, tissues are highly metabolically active [4]. Pancreatic tissue is one of the most difficult because of high activity of enzymes including triacylglycerol lipase, phospholipase A_2, cholesterol esterase, or galactolipase. Therefore, the aim of this study was to develop a preanalytical tissue processing workflow to minimize lipolytic enzyme activity. During the optimization, the effects of sample freeze-drying, tissue cutting and weighting temperature as well as sample disintegration solvent were taken into account. The most preserved conditions, with no processing time-dependent increase in the concentrations of hydrolysis products, required the employment of liquid nitrogen and the processing of deep-frozen tissue until the sample was immersed in the extraction solvent. Finally, the optimised protocol was applied to compare porcine, mouse, and human pancreatic lipidomes taking into account glycerolipids, glycerophospholipids, sphingolipids, cholesterol, and its esters.

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Acknowledgment

Development of RP-UHPSFC/MS Method for Determination of Nonpolar Lipid Classes in Biological Samples

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P14 **PRIZE**

Lipidomics is the field related to the determination of lipids in biological systems. The term lipid includes a large group of compounds, generally soluble in organic solvents, which are chemically and structurally heterogeneous. Their groups consist of nonpolar lipids, forming important biomolecules crucial for energy production and storage in all cells. They also play an important role in cellular homeostasis, however, the importance of individual lipids in these processes is not fully understood. Their analysis is not simple. Therefore, highly effective methods that can distinguish individual lipids side by side in biological matrices are required. For this reason, lipid analyses are based on mass spectrometry (MS) either with direct infusion (shotgun) or in connection to prior separation. A very advantageous combination for the analysis of nonpolar lipids is reversed-phase ultrahigh-performance supercritical fluid chromatography (RP-UHPSFC), which can be easily combined with electrospray ionization MS. RP-UHPSFC can separate lipids into lipid classes according to their polarity and also into lipid species according to the length of the fatty acyl chains and the number and position of the double bonds.

The main task of this study was to develop high-throughput RP-UHPSFC for the determination of nonpolar lipid classes, namely, fatty acids, sterols, sterol esters, mono-, di-, and triacylglycerols in plasma or serum samples. RP-UHPSFC method using sub-2-mm particles columns was coupled to high-resolution mass spectrometry using a quadrupole – time of flight (QTOF) mass analyzer and electrospray ionization (ESI). Full scan and MS/MS spectra were measured in positive-ion mode and identification of individual lipids was based on retention of behavioral characteristics. In total, 389 nonpolar lipids were identified in human serum/plasma samples.

UHPSFC/MS Lipidomic Quantitation of Porcine and Mouse Liver Tissues

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P15

The accurate quantitation of lipids in the blood of cancer patients can improve disease diagnosis and early treatment, ultimately improving the patient prognosis. Mouse models are commonly used to understand the biological mechanism behind the dysregulation of lipids and metabolites. However, porcine morphology and metabolism are closer to humans than mice. Reliable, accurate, and high-throughput methods are required for the quantitative analysis of large sample sets in current lipidomics.

We optimized a lipid extraction protocol based on the Folch method to accurately quantify the lipidome of porcine and mouse liver tissues. Our validated method based on ultrahigh-performance supercritical fluid chromatography (UHPSFC) coupled to electrospray ionization high-resolution mass spectrometry (ESI-MS) was applied.[1] The lipidomic strategy consisted of lipid class separation using two internal standards per lipid class. LipidQuant 2.1, an open-source software for the identification and quantification of lipids, allows type I and type II isotopic correction to achieve accurate concentration values.[2] In addition, plasma samples were analyzed to evaluate this biofluid as a mirror of liver tissue in our experimental models. Similarities and differences between organisms and sample types are evaluated based on total lipid class concentration, fatty acyl chain length, and double bond number composition of lipid species. Challenges and pitfalls during the sample processing regarding accurate lipid quantitation are presented.

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Interpretable Lipid Structure and Network Analysis

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P16 PRIZE

Graph-based algorithms are nowadays in the standard repertoire of computational genomics, transcriptomics, and proteomics workflows. However, comparable tools are missing from the landscape of lipidomics data analysis. Due to the growing importance of lipidomics for clinical and biomedical research, improvements in the interpretation of lipid data can directly translate into higher clinical relevance of lipidomics data.

To leverage the potential of lipid metabolic networks, we developed a framework called LINEX2 (Lipid Network Explorer 2) [1]. It generates lipid networks using curated information on lipid-metabolic reactions from public databases together with information on fatty acid metabolism. The resulting networks are not only on a lipid species level, but also specific to the lipids measured in a given lipidomics dataset. Additionally, LINEX2 supports user contributions for new lipid classes and metabolic reactions to keep up with novel insights into lipid metabolism in a community effort.

Since enzymes participating in lipid-metabolic reactions are multispecific, meaning one enzyme can catalyze the same reaction for different combinations of lipid species, an analysis of lipid-metabolic networks should take this peculiarity into account. Therefore, we developed an enrichment algorithm, specifically designed for this setting. By inferring changes in enzymatic activity considering the context of their multispecific nature and identifying a subnetwork of maximum change, it aids the mechanistic interpretation of quantitative lipidomics data.

As a proof-of-principle for the enrichment algorithm we analyzed data from a study by Thangapandi et al. [2], which compares the lipid profile of the liver between wild-type mice and mice with a hepatospecific knock-out of MBOAT7 under non-alcoholic fatty liver disease conditions. As the reaction catalyzed by MBOAT7 is already known, such a dataset is well-suited for testing the LINEX2 enrichment algorithm.

The subnetwork resulting from the analysis pinpoints the reaction catalyzed by MBOAT7, demonstrating the capability of our framework to infer changes in reaction activity on the basis of lipidomics data.

To showcase how our analysis facilitates hypothesis generation on real-world clinical data, we also applied the LINEX2 analysis workflow to data from the AdipoAtlas [3], a comprehensive reference set of the White Adipose Tissue lipidome of lean and obese humans.

The enrichment results indicate that the highest change in reaction activity is occurring in reactions catalyzed by the Phospholipase A2 Group IVC (PLA2G4C) and the asparaginase. These results are also supported by literature, which reports PLA2G4C to be differentially expressed in obese individuals and products of PLA2 activity to be mediators of adipose tissue metabolism.

LINEX2 is available as a web service (https://exbio.wzw.tum.de/linex2/) and a python package for high-throughput analysis (https://pypi.org/project/linex2/).

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[2] Thangapandi et al., 2021, Gut
[3] Lange et al., 2021, Cell Reports Medicine

Introducing the Lipidomics Minimal Reporting Checklist

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P17

The number of lipidomics experiments is rapidly growing in the previous 10 years and so does the frequency of published articles in scientific articles describing these experiments. However, many publications do not provide a complete or transparent set of information to either comprehend, reproduce, or even reuse the data or results from these experiments. These reports may lack a controlled vocabulary, a clear structure, or even no access to the raw data. Another issue emerges when non-lipidomics specialized journals send articles for peer review to non-domain experts for a sophisticated assessment of the described methods.

In a community-wide effort, we implemented an interactive lipidomics reporting checklist [1] to overcome these limitations. The Lipidomics Standards Initiative (LSI) compiled guidelines for minimum reporting standards with the assistance of the lipidomics community of the International Lipidomics Society (ILS). The interactive checklist is a web-based questionnaire [2]. Users can select several lipidomics methods such as *direct infusion* or *separation* to customize their report. The checklist guides the user through eight sections, i.e., *Overall study design*, *Preanalytics*, *Lipid extraction, Analytical platform, Lipid Identification & Quantification, Quality control, Method qualification and validation, and Reporting.* Each section demands a minimum set of information in order to continue. The resulting pdf report can further be, e.g., attached as supplementary to an article, and published separately via the Zenodo platform. This system intends to support analytical chemists to ensure that their provided data are of the highest level and educate young scientists in this field to learn the crucial requirements within this discipline. The questions in the checklist provide profound explanations and link to literature for further details. The checklist is free of charge and can be used without any limitations.

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A targeted LC-MS/MS method for comprehensive, quantitative analysis of bioactive lipids

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P18 **PRIZE**

Bioactive lipids govern many cellular processes, including cell growth, death, and migration, and are key players in inflammatory processes. [1-2] While approaches have been made to develop an assay covering a few of these lipid classes [2], there is yet no comprehensive workflow that enables the quantification of all relevant lipid classes in one analytical run. Challenges include a high dynamic range and, most of all, these analytes' very different structures and chemical properties. [2]

Analytes from 15 different lipid classes were selected to establish an LC-MS/MS method on a QTrap 6500+, including eicosanoids, fatty acids and conjugates, fatty amides, sphingoid bases, ceramides, and lyso species of glycerophosphatidylserines, glycerophosphatidylinositols, and phosphatidic acids. Different UHPLC columns, solvent compositions, and additives were tested. Chromatographic and MS source parameters were optimized to ensure the highest selectivity and sensitivity of the final method.

Column selection was the most critical step during method development. All tested columns shared the same dimensions and particle sizes but the achieved resolution differed greatly. Most importantly, only one specific column enabled the separation of all 15 lipid classes with good performance in terms of signal intensity, chromatographic separation, and peak shape. Apart from the column type, solvent composition proved to be the most important variable for obtaining a robust and sensitive LC-MS/MS method. We herein report the first LC-MS/MS method that enables the simultaneous measurement of 15 different bioactive lipid classes in one LC-MS run, including challenging lipids like lysophosphatidic acids and sphingosine-1-phosphates.

Literature:
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[2] Schoeman, J. C. et al, Development and application of a UHPLC-MS/MS metabolomics based comprehensive systemic and tissue-specific screening method for inflammatory, oxidative and nitrosative stress, Analytical and Bioanalytical Chemistry **2018**, 410 (10), 2551-2568.

High-Throughput and Comprehensive Lipidomic Analysis of Human Plasma Using UHPSFC/MS

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P19　**PRIZE**

Lipidomics studies the structure, function, and metabolism of lipids in living systems, where they play essential roles in cellular processes, such as energy storage, signaling, and membrane structure. The combination of chromatographic technique and mass spectrometry enables the identification and quantitation of hundreds of lipid species, including various forms of isomers. The modern trend in lipidomic analysis is fast analysis and high separation efficiency, enabled by columns with sub-2 μm particles as the stationary phase. The critical step of the lipidomic analysis is the sample preparation. The liquid-liquid extraction allows purification of the sample from impurities and salts, but analytes can be split into both phases due to the diverse character of lipids.

Ultrahigh-performance supercritical fluid chromatography coupled to mass spectrometry with high-resolution (UHPSFC/MS) was used for identification and quantification. The Acquity BEH UPC2 (100 × 3 mm, 1.7 μm, Waters) column was employed for the separation of lipid (sub)classes, due to the coelution of the internal standards and the analytes guaranteeing the same matrix effect. The sample preparation was performed by precipitation of proteins using a mixture of butanol/methanol, which enables the extraction of polar and nonpolar lipids together in a high yield. The method was used for the identification of lipids in human plasma measured in positive and negative ion modes (accurate mass lower than 10 ppm) and retention dependencies. The method was validated for human plasma using at least two internal standards per lipid class, including calibration curves, LOD, LOQ, matrix effect, selectivity, *etc.* The full validated method was used for quantification of lipids in NIST human plasma and concentrations were correlated with reference values. The improvement of the method brings the identification and quantification of more lipid classes/species and the determination of accurate concentration by our LipidQuant 2.1 using both type I and type II isotopic corrections.

LC-MS investigation of artificially oxidized phosphatidylethanolamines in Saccharomyces cerevisiae based on two-dimensional heart-cut liquid chromatography

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P20 **PRIZE**

Lipid (per-) oxidation as a hallmark of oxidative stress is closely related to many diseases and hence a point of interest in current research. Double bonds of unsaturated fatty acids are most susceptible to lipid (per-) oxidation. Fatty acids are primarily contained in more complex lipids like phospholipids (PL) and can be classified into mono-/ polyunsaturated (MUFAS/PUFAS) and saturated fatty acids defining their oxidative susceptibility. In human cells PL are the most abundant lipid class, each subclass found to be responsible for different biological functions. As model organism with a well-known PL composition, *Saccharomyces cerevisiae*, known as baker's yeast, was chosen resembling the human lipidome.

Due to their relatively high abundance in human cells and importance as one of the main membrane lipids, (per-) oxidated phosphatidylethanolamine (PE) species were examined more closely. The naturally low abundance and chemical properties of (per-) oxidated lipid species requires the use of powerful hyphenated chromatographic and mass spectrometric techniques. For lipid extraction, an established protocol according to MATYASH *et al.* was conducted.[1] Peroxidation was achieved by means of Fenton reaction and parts of the solution were subsequently reduced with triphenylphosphine (TPP) to obtain the respective hydroxy species for additional measurements. For chromatographic separation of these highly complex lipid species, a two-dimensional *heartcut* liquid chromatographic technique was developed. The unique molecular features of each PL subclass, defined by their respective headgroup, enable class-specific separation utilizing hydrophilic interaction liquid chromatography (HILIC) in the first dimension. This allows for an exact *heartcut* of the PE fraction also including (per-) oxidated species which are solely transferred onto a short C18 trapping column for focusing and cleaning prior to analysis in the second dimension. Here a reversed-phase (RP) separation with an orthogonal selectivity is performed. (Per-) Oxidated and non-oxidated PE species are separated according to their respective fatty acid moieties. The chromatographic separation is followed by mass spectrometric detection with a high resolution (HR) mass spectrometer (MS) and MS/MS experiments for species identification. For reliable identification of each PL species, all available information like retention times, exact *m/z* ratios and fragments generated by MS/MS experiments were considered.

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New method to characterize epidermal Ceramides by Supercritical Fluid Chromatography (SFC) coupled to High Resolution Mass (Q-Tof): application to various samples with skin barrier default.

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P21

Lipids are the main constituent of skin and epidermis, and are mainly constituted by ceramides with prevalent very long acyl chains, free fatty acids, and cholesterol. In health context, the lipid composition of skin can be highly modified in diseases thus its full characterization is essential. Epidermal ceramides, specificaly ω-O-Acylceramide, will have a crucial role for skin barrier function. The epidermal ceramides is a large and complex family of 12 sub-classes and their structural diversity, their wide range of concentration and the few standards available make this analysis very challenging.

The aim of this work was to develop an original profiling of the epidermal ceramides using supercritical Fluid Chromatography (SFC) (*UPC² Waters)*, which is a technology especially suitable for the non-polar metabolites, coupled to a Q-TOF (*Xevo, Waters*). The profiling was developed on a Torus DIOL column with a gradient of a mixture of isopropanol, methanol and acetonitrile (0.1% formic acid) in CO_2 under pressure with ionisation in negative mode in presence of methanol. In studies involving the skin we can find many samples: it can be whole skin biopsies, cigarette paper impregnated with sebum, strip, solvent-in vivo skin extraction but also reconstructed human epidermis (RHE) for *in vitro* skin studies. Sample preparation needs to be adapted to sampling. We will present the development and the validation of this new method from the sample preparation to the data treatment to the chromatographic separation and structural characterisation.

Mutation in patatin-like phospholipase domain-containing 1 (PNPLA1) causes autosomal recessive congenital ichthyosis. This mutation conducts to lethal phenotype with major defects in the epidermal barrier specially a blocage in ω-O-acylceramide synthesis. We will show the first application of the developed ceramide profiling on mutated Pnlpa1 mice and RHE samples.

Description of FAHFAs in Caenorhabditis elegans

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P22

The biomedical model organism *Caenorhabditis elegans* (*C. elegans*) harbors a rich metabolome and lipidome and new classes of molecules are described on a routine basis. Several peculiarities make the *C. elegans* lipidome different compared to e.g. mammalian lipidomes. Here, we describe the detection and identification of novel Fatty Acyl esters of Hydroxy Fatty Acids (FAHFAs) in *C. elegans*.

FAHFAs have been originally described in adipose tissue and they are reported to correlate with insulin sensitivity. Typically, these lipids contain a long chain hydroxy fatty acid, e.g. C16 or C18 fatty acids. However, in contrast to this, all species identified in *C. elegans* contain a C9 hydroxy fatty acid. Interestingly, in ascarosides, an important class of signaling molecules in *C. elegans*, the most abundant species also contain C9 hydroxy fatty acids.

Our initial identification is based on library matching of tandem MS spectra, followed by the analysis of retention time trends. For several species multiple chromatographically separated peaks could be detected. Based on these results, we generated an *in-silico* library to improve the annotation of FAHFAs. Ongoing efforts aim to identify the position of hydroxyl groups and further species of FAHFAs in *C. elegans*.

Profiling of lipids in human plasma with Total Correlation–Mass Spectrometry (TOC-MS)

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P23

When profiling lipids in a complex matrix like human plasma, it is important that scientists have access to robust analytical techniques, which accurately identify lipids to at least the molecular species level. Traditionally, this has been achieved with LC-MS/MS based approaches but poor separation, drifting retention times and limited structural information from fragmentation hinder lipid identification.

Total Correlation-Mass Spectrometry (TOC-MS) is a new MS/MS technique that allows unambiguous correlation of molecular fragments and precursors in a single rapid experiment. Combined with ultraviolet photodissociation (UVPD), TOC-MS produces more structurally informative fragments to aid in lipid identification, including alkyl chain composition and double bond position. By eliminating chromatography, TOC-MS reduces the amount of method development, sample preparation and data standardisation required to achieve consistent and informative results.

Desalted human plasma was purchased from Sigma Aldrich and lyophilized in 1 mL aliquots. Lyophilized plasma was reconstituted in a 1:1:1 solution of H2O:MeCN:IPA with 0.1% formic acid and 40 mM ammonium bicarbonate, and spiked with 100 nM of SPLASH Lipidomix (Avanti). Once reconstituted, samples were infused into the TOC-MS instrument at a flow rate of 2.5 µL min-1 and ionised using ESI in positive ionisation mode. A 213 nm UV laser was used to perform UVPD. Data was analysed using custom software and lipid precursors were identified using available lipid databases and prior publications. High resolution MS and UVPD fragmentation data was then used for lipid identification.

As part of data processing, the results were peak picked to collate a list of 76 potential lipid precursors with an intensity greater than the mean. From database searching this corresponded to 276 lipids from the ALEX123 lipid calculator and 568 lipids from Lipid Maps. Within this list of potentially detected lipids, were twenty-two phosphatidylcholine (PC) and lyso-phosphatidylcholine (LPC) lipids that had been identified in literature. For each of the potential PC or LPC lipids, a MS/MS spectrum was extracted, which contained a minimum of two fragments, corresponding to the PC headgroup and the corresponding counter fragment. An additional feature of TOC-MS is the ability to search for precursors that share a common fragment, or a common neutral loss. By searching for the PC headgroup fragment, five low abundance precursors were identified.

This fragment information was used to confirm the molecular species of most lipids, but was not sufficient to distinguish between lipid isobars without additional fragments. Additionally, many of the precursors contained at least one double bond, with several containing multiple. By using UVPD with TOC-MS, additional fragments were produced that distinguished between isobars and identified double bond positions. This level of structural information is important in correctly identifying lipids when profiling human plasma and for understanding a lipid's biological function.

Lipidome-wide association study across adipose tissue, liver and skeletal muscle: the effect of diet and bile acid metabolism

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P24 — PRIZE

The organismal lipidome is remarkably diverse and its composition and abundance are determined by tissue-specific *de novo* synthesis and metabolization of external lipid sources, mainly from food intake[1]. Lipid profiles were shown to be organ-specific[2], however, the relative abundance and diversity of lipid species were not comprehensively investigated regarding the tissue function, effect of diet, and bile acid levels. Bile acids have been recognized as enterohepatic-derived hormones which control lipid metabolism and global metabolic health[3]. In the present study, we first evaluated the relative distribution and diversity of lipid species across four organs playing a central role in lipid energy metabolism (including lipid synthesis, utilisation, and storage): subcutaneous white adipose tissue (scWAT), brown adipose tissue (BAT), skeletal muscle (i.e., quadriceps) and liver. Furthermore, we aimed to determine the effect of diet on lipid composition of specified organs obtained from C57BL/6J mice fed with chow diet (CD) and high-fat diet (HFD). The mice (all males) were sacrificed at 29 weeks of age, following a period of 21 weeks on CD or HFD. The sacrifice was performed in the postprandial state (4h after physiological refeeding)[4]. Tissue lipidome was characterized using a high-coverage, highly sensitive and specific targeted SRM-based methodology coupled to HILIC separation mode[5]. The sample preparation workflow consisted of tissue lysis and single-step extraction with isopropanol[6]. More than 2400 lipid species were screened in the initial qualitative analysis (in multiple runs) followed by quantification of robustly detected species. Lipid quantification was achieved by a single point calibration with 75 isotopically labelled standards representative of different lipid classes, covering lipid species with diverse alkyl chain lengths and unsaturation degrees. Correction of isotopic overlap was performed using LICAR (https://slinghub.shinyapps.io/LICAR/). As a result, a wide panel of 24 lipid classes comprising 533 to 838 lipid species were quantified (CV<20%) depending on tissue type. Additionally, bile acids (15 species) were quantified in mice liver and plasma using stable isotope dilution approach[4].

The highest diversity of lipids was detected in quadriceps (n=838 species) while white adipose tissue contained the lowest (n=533 species) number of species. Main differences were found for triacylglycerols (TG) and glycerophospholipids such as glycerophosphocholines (PC) and glycerophosphoethanolamines (PE). In mice fed with HFD, we observed the accumulation of TG as a general trend for all tissues. Besides, a decreasing trend of some hexosylceramides (HexCer), and sphingomyelins (SM) was reported in a tissue-specific manner. The dataset is currently under investigation to identify the lipid signatures associated with measured physiological parameters including body and organ weight, blood glucose and bile acid levels.

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6. *Metabolites* **10**, 1–17 (2020).

4D – lipidomic protocols for deep coverage and routine profiling of plasma and tissue lipidome in human and animal model samples

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P25

Lipids play essential roles in many physiological processes and pathological conditions and serve as important candidates for therapeutic agents, biomarker discovery, treatment response, and follow-up monitoring. Successful translation of lipidomics from academic laboratories in clinical research requires still extensive optimization/adaptation of protocols to allow the confident, routine, and high-coverage lipid identification and quantification in biospecimens.

Implementation of lipidomics operational workflows in clinical profiling requires high-throughput extraction. Untargeted high-resolution trapped ion mobility mass spectrometry (tims)- based 4-dimensional lipidomics encompassing automated lipid extraction, 4-dimensional feature extraction, and cross-validated quantification strategy can expedite clinical profiling.

Here, we show the 4-dimensional tims- based confident lipidome phenotyping in plasma samples and mouse tissues. Furthermore, we investigated the performance of different quantification strategies such as normalization via level-3 standards as well as one-point and multi-point quantification in positive and negative ion modes for various lipidome phenotype cohorts.

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Lipidomic Test for Early Diagnosis of Pancreatic Cancer – Transfer to Clinical Practice

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P26

Pancreatic ductal adenocarcinoma (PDAC) has one of the worst prognoses among solid malignancies. The main reason is the late diagnosis due to the lack of symptoms at an early stage. Moreover, no screening program for PDAC is available. A new method for the early diagnosis of PDAC was developed at the University of Pardubice [1]. The diagnostic test is based on accurate and high-throughput lipidomic quantification. First, the lipidomic profiles of cancer patients and healthy controls are measured. Then, multivariate data analysis (MDA) is used to create orthogonal partial least squares (OPLS) models for differentiating patients and controls, which can be applied to samples with unknown classification. The whole process is patent protected, but the new company Lipidica (Lipidomic Diagnostics of Cancer) is the user of the patent and works on its transfer to clinical practice as the Lipidomic Diagnosis of Pancreatic Cancer ("LDPC test") used for PDAC screening. The lipidomic analysis is performed by ultrahigh-performance supercritical fluid chromatography coupled with high-resolution mass spectrometry (UHPSFC/MS). The lipids are extracted by Folch extraction, and the high-throughput UHPSFC/MS method [2] allows the quantification of lipid species from 8 lipid classes in a total run time of 8 min including column equilibration. The methodology (analysis, data processing, and multivariate statistical analysis) was transferred successfully from the University of Pardubice to Lipidica, which was performed based on the lipidomic analysis of the same plasma samples from females and males in both places, resulting in the same lipidomic differences between females and males investigated by MDA. Furthermore, the precision of the test based on biological material was investigated for more than 150 cancer patients and 200 healthy control samples of plasma and serum. The transfer of methodology and the selection of the biological material were the first steps of the LDPC test on the way to clinical practice. The next step is clinical validation that focuses on high-risk groups of people for the outbreak of PDAC (i.e., the lifetime risk of PDAC is higher than 5%). The result of LDPC test will be correlated with current diagnostic approaches (endoscopic ultrasound and magnetic resonance imaging). After successful clinical validation of LDPC test, the method could be implemented in clinical practice and later in the national screening program for PDAC.

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Utilising the total phosphorus content as a normalisation strategy in quantitative lipidomics studies

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P27 · PRIZE

Ensuring comparability between samples in lipidomics studies possesses many challenges and while for some matrices like plasma and serum feasible normalisation approaches are available, for other matrices often a suitable normalisation strategy is not present. In addition to using the net volume for liquid samples (e.g. plasma), many studies utilise the net weight or the protein content as a reference. Especially the last two methods also bear disadvantages that affect comparability, e.g. is the net weight dependent on the water content of the sample which can vary largely. Here, we present a normalisation method utilising the total phosphorus content as a reference value to employ in the quantitation of phospholipids in biological samples. The nematode *Caenorhabditis elegans* was chosen as a model for this study, because up to now there is no suitable normalisation strategy for lipid studies working with this organism. Studies utilising the worm often include the work on cardiovascular or neurodegenerative diseases and ageing. In the here presented normalisation approach, the lipids first were extracted with a protocol according to Folch *et al*.[1] Afterwards, part of the extract was digested with nitric acid and the total phosphorus content was determined by inductively coupled plasma-optical emission spectroscopy (ICP-OES). The other part of the lipid extract was used for lipid quantification via supercritical fluid chromatography hyphenated to trapped ion mobility spectrometry-mass spectrometry (SFC-TIMS-MS). The phospholipids were separated according to their respective lipid class via SFC and identified with the accurate mass, retention time and MS/MS experiments. The quantification of phospholipids was carried out with a coeluting deuterated internal standard via the ion mobility signal of the TIMS dimension. A combination of the elemental and molecular information yielded less variance in the concentrations of the quantified phospholipid species and an improved comparability of the biological samples proving this method effective.

[1] Folch, J.; Lees, M.; Sloane Stanley, G. H. (1957): A simple method for the isolation and purification of total lipides from animal tissues. In: *The Journal of biological chemistry* 226 (1), S. 497–509.

The significance of de novo lipogenesis in platelets

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Background. Blood platelets are essential in mediating hemostasis in response to injury. Most of their functions are largely dependent on the composition of the plasma membrane, which plays an essential role in membrane fluidity and curvature, platelet shape change and the generation of bioactive signaling molecules. Platelets have the ability to synthesize phospholipids and fatty acids de novo. They express acetyl-CoA carboxylase 1 (ACC1), a key enzyme that regulates lipid metabolism as it catalyzes the production of malonyl-CoA, the rate-limiting substrate for de novo lipogenesis.

Aim. To investigate the impact of ACC1 deficiency on platelet lipidome, and the subsequent alteration of platelet shape, activation, and thrombus formation.

Method. We generated a Cre transgenic mouse strain that allows specific deletion of ACC1 in megakaryocytes/platelets (GpIbCre$^{+/-}$ x ACC1$^{flx/flx}$, pKO ; GpIbCre$^{+/-}$, CRE). Lipidomic analysis was performed in resting platelets using the commercial Lipidyzer platform. Microscopy, flow cytometry and biochemical assays were used to examine structural and functional changes in mouse platelets. Thrombus formation was assessed in vivo by intravital microscopy.

Results: Lipidomic analysis identified 468 lipid species belonging to 17 different lipid classes. A total of 174 lipid species, representing 37% of the platelet lipidome analyzed, showed differential regulation between the two genotypes. Class enrichment analysis indicated that ACC1 deficiency resulted in enrichment of downregulated lipids in the phosphatidylethanolamine plasmalogen (PEP) lipid classes, while up-regulation was observed in the ceramides (CER), phosphatidylethanolamine (PE) and phosphatidylcholine (PC) lipid classes. Moreover, ACC1 deficiency was associated with a change in the fatty acyl chain length. It decreases short satured fatty acid and increases long polyunsaturated fatty acid (PUFA), including arachidonic acid (AA, C20:4), eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6) contained in PC and PE. Interestingly, ACC1 pKO platelets showed a significant decrease in all AA-containing PEPs. These changes in the platelet lipidome were associated with structural modifications and altered platelet activation and thrombus formation.

Conclusion: ACC1 is a tight regulator of specific lipid pools required for platelet shape change and thrombus formation. These results make this enzyme a potential new target for anti-thrombotic pharmacological strategies.

Mass spectrometry for monitoring and quality control of differentiation of pluripotent stem cells to lung progenitors using biostatistical models

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With increasing demands on precise analyses of biological samples in complex biological matrices, there is also need to develop and optimize mass spectrometric (MS) methods. The whole cell MALDI TOF MS is already used in clinical microbiology and diagnostics. In recent years it has been introduced also to cell biology, immunology, and cancer biology. Recently we used the whole cell-MS to monitor cultures of stem cells and progenitors and elucidate phenotypic shifts in long-term cultures. Here we demonstrated precise tracking of differentiation trajectory of human embryonic stem cells (hESCs) to lung epithelial progenitors by whole cell MS coupled with biostatistical modelling.

Human embryonic stem cells (hESCs) possess unlimited differentiation potential and capacity to self-renew indefinitely. The hESC-derived, expandable lung epithelia (ELEP) used in this study were recently established in our lab to address histogenesis and regeneration of functional lung cell types. Differentiation of hESCs towards ELEPs is a complex process that shows substantial heterogeneity and can also produce aberrant cells with unwanted properties, such as lack of functional phenotype, or propensity to cancer growth. The differentiation process can be outlined specifically by molecular markers, but an unbiased, sensitive, and robust tool for the discrimination of ELEPs from pluripotent or transitional stages is still missing.

In this work, we optimized the whole cell MS for lipid analysis, and coupled it with the multivariate statistical methods and supervised methods based on machine learning to follow differentiation of hESCs to ELEPs. We visualized the full differentiation trajectory based on spectral data only and revealed also some phenotypic abnormalities linked to passage number, and by proxy aneuploidy status of hESCs.

Various extraction methods were tested to monitor changes in cellular lipids during the differentiation process. Finally, Folch´s method using chloroform/methanol/water has been selected and followed through this work. Sinapinic acid and 9-Aminoacridine matrix were used for MS measurement. MS measurements were combined with in-house developed R scripts. Data obtained from mass spectra were analyzed via several methods including principal component analysis (PCA), heatmap, and boxplots. Data were also analyzed by supervised methods (decision tree, random forest, and artificial neural networks). Mass spectra at various differentiation stages revealed different spectral fingerprints which allowed for successful classification in mathematical space using PCA and others. In summary, whole cell-MS is a promising tool for complex cultures of hESC-derived lung cells and progenitors, with potential clinical translation.

Quantitative Analysis and Lipidomic Profiling of Human Serum of Pancreatic Cancer Patients Focused on Less Abundant Lipid Classes by HILIC-UHPLC/MS

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Lipidomics is a field that focusses on the study of lipids in living systems. Lipids play an important role in all organisms, such as membrane components, energy storage, precursors for metabolic processes, and signaling, which predestines lipids for biomarkers of cancer, cardiovascular diseases, and neurodegenerative diseases. The comprehensive analysis of high- and low-abundant lipid classes together is extremely challenging because injection of concentrated extract leads to the saturation of the detector, ion suppression, or contamination of the mass spectrometer, while signals for low-abundant lipids are missing in diluted extracts. Moreover, the selective extraction is almost impossible because of the structural similarities. However, the analysis of all lipid classes within their biosynthetic pathways is essential for understanding lipid dysregulations in human metabolism caused by various diseases, such as cancer.

Hydrophilic interaction liquid chromatography separates lipids according to the polar head group, which allows the switch of highly abundant lipid classes to waste, which enables the injection of concentrated lipidomic extract. An Agilent 1290 Infinity series LC coupled to a Xevo G2-XS QTOF mass spectrometer was used and the new separation method using the Type-C Cogent Silica column (150×2.1 mm; 2.1 μm) column with the total run time of 20 min was developed. Deproteinization using a mixture of BuOH/MeOH was used for the sample preparation. The method is based on the switch of inorganic salts in the void volume and high-abundance lipid classes (cholesterol esters, triacylglycerols, and phosphatidylcholines) to the waste, preventing contamination of the mass spectrometer. In total, 246 lipid species from 24 lipid subclasses using both positive and negative ion modes were identified in pooled human plasma. The method was fully validated and almost two hundred lipids were quantified using an internal standard for each lipid class. Our laboratory-made software LipidQuant 2.1 was used for semi-automated quantitation of lipid species. The method was used to investigate the lipidomic profile of serum samples obtained from patients with pancreatic cancer (PDAC) and healthy controls, which revealed statistically significant differences in lipid concentration. Statistical projection methods, for example, PCA, OPLS-DA, S-plot, box plots, and network maps, are used for the visualization of results. In PDAC samples, we observed significant down-regulation of sphingolipids (SL) with very-long *N*-acyl chains, together with up-regulation of SL with shorter *N*-acyl chains. These dysregulations are observed in the whole metabolic cascade of SL from ceramide to glycosylated ceramide, indicating the metabolic disorder in the synthesis of ceramide caused by cancer. Moreover, the dysregulation corresponds with bonding of fatty acids, where phosphatidylethanolamines (PE) with acyl bonds are upregulated, while PE with ether/plasmyl bond are downregulated.

Serum Lipid Profiles in relation to Clinical Presentation and Severity of Coronary Artery Disease: Preliminary Results

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Atherosclerosis is a pathological process strongly correlated with lipid dysmetabolism and oxidative stress. Accompanying inflammatory alterations on the endothelium can further lead to coronary artery disease (CAD), including stable and acute coronary syndrome. Among the potential molecular mechanisms, lipids are of central importance for the short-term metabolic flexibility of the heart, and lipotoxic compounds could be a key factor linking metabolic stress to persistent damage in myocardial tissue. The "CorLipid" study (NCT04580173) is an investigator-initiated, prospective, non-interventional cohort trial involving patients undergoing coronary angiography. In this study, we selected a subgroup of 146 enrolled patients with acute coronary syndrome (ACS, n=48), chronic coronary syndrome (CCS, n=52), and healthy individuals (n=46), to investigate the lipid profile. Serum samples were extracted with MTBE:MeOH and lipid extracts were analyzed by reversed-phase liquid chromatography (Vanquish Horizon) coupled with an Orbitrap Exploris™ 240 Mass Spectrometer (ThermoFisher Scientific, Germering, Germany). Lipids were identified using two software tools: LipidHunter2 and Lipostar2 (Molecular Discovery Ltd). Lipid annotations were controlled by plotting the retention time of a given lipid species against its Kendrick mass defect (KMD) to the hydrogen base. LC-MS data were quantified in Skyline and type I isotopic correction was performed. In total, we identified 448 lipids representing the major lipid subclasses. Preliminary multivariate and univariate statistical analyses have shown discrimination between the serum lipidomes of ACS patients and healthy individuals. The levels of various glycerophospholipid and sphingolipid species were significantly different in patients with CAD. Overall, 49 lipids were found to be significantly altered, including 1 LPC P, 2 PC O, 1 PC P, 8 PCs, 6 PEs, 1 PI, 6 DGs, 10 TGs and 14 SMs. These findings provide molecular insights into the role of dysregulated lipid metabolism in the development and progression of CAD, and their investigation could open novel and promising therapeutic strategies to improve the classification of CAD phenotypes.

Novel omic Mass Spectrometry (MS) data analysis software applied untargeted LC-MS/MS lipidomics use

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Metabolomics is a comprehensive analysis of metabolites produced by cellular activities, and analysis using mass spectrometry is essential. Especially in the search for unexpected molecules in lipidomics, where molecular diversity is high, the use of un-targeted analysis with high coverage is preferred over selected ion monitoring (SRM) analysis, which can measure target molecules with high sensitivity. However, the large size of un-targeted analysis data has been a concern for researchers because of a lot of time and effort required from peak extraction to quantification. We have developed an integrated lipidomics analysis system (MetaboAlign (MA)) that can automatically perform peak extraction, peak alignment, peak quantification, and annotation of fatty acid molecular species with high speed and high precision using FT-MS data.

Samples of Folch monophasic lipid extract from mouse brain homogenates were separated by UHPLC (Vanquish) using a C18-1.7um reverse phase column, and FullMS-IDA measurements were performed using high-resolution FT-MS (Q Exactive HF), and the acquired MA analysis was performed on the acquired data. Lipid Search v5.1 was used for identification of fatty acid molecular species.

When 1684 lipid molecules were registered for 4 samples of negative ion mode data with a file size of about 200 MB per sample and processed with a mass error of 3 ppm or less, the process took 20 min from data registration to peak extraction and 3 min from peak alignment to peak quantification and identification, extracting about 3000 peaks and identifying about 700 molecular species.

This software is vendor-free and can analyze data from various LCMS measurement methods such as PRM method, and is expected to be used not only for Lipidomics but also for Metabolomics and Proteomics in the future.

Ether lipid modulation in a preclinical model of dilated cardiomyopathy remodels the cardiac and circulating lipidome and attenuates pathological cardiac morphology

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INTRODUCTION: Plasmalogens are ether phospholipids abundant in several tissues including the heart. Plasmalogen levels are known to be dysregulated in a variety of disease settings, including neurological, metabolic and cardiovascular diseases. Modulation of plasmalogen levels via dietary supplementation of its precursors called alkylglycerols (AKG) have shown to be protective in mouse models of atherosclerosis, however its therapeutic potential in a setting of heart failure is still unknown.

AIM: To assess if AKG supplementation in a transgenic mouse model of dilated cardiomyopathy (DCM) can increase cardiac plasmalogen levels and attenuate cardiac pathology.

METHOD: ~10 week old non-transgenic (Ntg) and transgenic mice that developed DCM through cardiomyocyte-specific overexpression of mammalian sterile 20-like kinase 1 began dietary supplementation of chow +/- 1.5% AKG (40% Chimyl Alcohol: 30% Batyl Alcohol: 30% Selachyl Alcohol) for 16 weeks (n=14-16/group). Mice underwent echocardiography to assess heart function at baseline and endpoint. Lipids were extracted via the chloroform: methanol method and run using liquid chromatography electrospray ionisation tandem mass spectrometry. Unpaired t-tests with Benjamini Hochberg corrections were utilised for lipidomic analyses, and 2-way ANOVA with Tukey's post-hoc was used for morphological assessments. $P<0.05$ was considered significant.

RESULTS: Heart and lung weight/tibia length ratios (HW/TL, LW/TL), both markers of heart failure, were significantly increased in DCM chow vs Ntg mice. AKG supplementation successfully increased total plasmalogen levels in the heart and circulation of Ntg (163.9% and 139%, respectively) and DCM (162.4% and 200%, respectively) mice vs chow controls. AKG supplementation in DCM mice was associated with a reduction in HW and LW/TL (17.1% and 19.1%, respectively). AKG supplementation in DCM mice also decreased several individual ceramide species, lipids that have been associated with heart disease, and inversely, increased tetra-linoleoyl cardiolipins (163%) that is known to be integral to mitochondrial function. In contrast, the phospholipid classes phosphatidylethanolamine, phosphatidylcholine and phosphatidylinositols were significantly decreased in the heart and plasma of AKG supplemented mice.

CONCLUSION: AKG dietary supplementation substantively remodels the cardiac and circulating lipidome, while successfully increasing ether lipids in mice that developed DCM. AKG supplementation was associated with an attenuation of pathological cardiac morphology. This was accompanied by a decrease in ceramide species and increase in tetra-linoleoyl cardiolipins, suggesting an improvement in overall cardiometabolic health. While AKG supplementation presents as a viable option as treatment for heart failure, additional work is required to elucidate the mechanisms by which plasmalogen modulation confers cardioprotection.

Analysis of esterified oxylipins in the membranes (oxidized phospholipids) by means of targeted and untargeted LC-MS/MS

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Eicosanoids and other oxylipins are oxygenated polyunsaturated fatty acids (PUFA) generated either enzymatically or non-enzymatically. Oxidized PUFA are present in biological samples in non-esterified form but most of them, especialy hydroxy-PUFA and epoxy-PUFA, are esterified to phospholipids in cell membranes or lipoproteins. Esterified oxylipins are commonly quantified following alkaline hydrolysis of the lipids (ProstagOth Lipid M, 2020, 146, 106384) as free i.e. non-esterified oxylipins. However, this indirect analysis using targeted liquid chromatography (LC) coupled to mass spectrometry (MS/MS) does not allow drawing conclusions to which lipids the oxylipins are bound.

In this study, the incorporation of oxylipins in the membranes was investigated by cultivating HEK293T cells in medium supplemented with non-esterified oxylipins. The oxylipins esterified in phospholipids were analyzed by untargeted LC-high resolution MS/MS following sonication of the cells and extraction of oxidized phospholipids. The untargeted approach allows to deduce the fatty acids/oxylipins esterified to the phospholipids as well its lipids class based on the elution time and the fragmentation spectra. Lipids bearing hydroxy-PUFA (e.g.15-HETE) and the corresponding epoxy-PUFA (e.g. 14(15)-EpETrE) cannot be distinguished based on their fragmentation spectra. However, as the species bearing epoxy-PUFA elute later than those bearing hydroxy-PUFA, a differentiation is possible.

The amount of esterified the oxylipins in the cells was indirectly quantified using a targeted oxylipin LC-MS/MS method. Samples were prepared by precipitation of proteins and the ester bond was cleaved by hydrolysis using potassium hydroxide. Non-esterified oxylipins were then extracted via solid-phase extraction (Bond Elut Certify II material). Parallel analysis of non-esterified oxylipins before and after alkaline hydrolysis reveals the portion of oxylipins bound to lipids.

For both methods, the chromatographic separation was carried out on RP-columns filled with sub 2 μm particles. Targeted oxylipin detection was performed using negative electrospray-ionization and selected reaction monitoring on a 5500 QTRAP mass spectrometer. For the untargeted lipid analysis, the ionization was carried out by a heated electrospray HESI-II in positive and negative mode. Mass spectrometric detection was performed using Full MS/data dependent MS2 enabling the acquisition of fragment spectra of regioisomeric oxidized lipids by means of high-resolution hybrid quadrupole-Orbitrap (Thermo Scientific Q Exactive HF).

The incubation of oxylipins in HEK293T allowed us to detect phospholipids bearing both hydroxy-PUFA as well as epoxy-PUFA and gained first insights in which lipid class the oxylipins are bound.

Quantitative and qualitative analysis of steroids by high- resolution mass spectrometry

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The work demonstrates the ability of the SCIEX ZenoTOF 7600 system to sensitively quantify steroids and to qualitatively characterize their structures using electron-activated dissociation (EAD)-based fragmentation. The high speed of the MS/MS acquisition (133 Hz) mode enables high sample through-put while maintaining good spectral quality. The complimentary EAD fragmentation mode provides diagnostic fragments to distinguish steroid isomers and isobars. Hormonal steroids regulate most body functions and the dysregulation of these molecules can play a role in the pathophysiology of human disease. Early techniques to measure endogenous steroids include immunoassay and gas chromatography-mass spectrometry (GC-MS). Immunoassays are problematic because they lack specificity for low-level steroids due to interference from endogenous steroids present at higher levels. In contrast, GC-MS offers higher specificity and is sensitive to low-level steroids, however, it requires extensive sample preparation via derivatization. More recently, steroid analysis by liquid chromatography-tandem mass spectrometry (LC-MS/MS) has emerged as a sensitive and specific technique with a simplified sample preparation.

Hormonal steroids were analysed by LC-MS/MS using the ZenoTOF 7600 system. All quantita-tive data were analyzed using the Analytics module in SCIEX OS software. Qualitative data were processed using the Explorer module in SCIEX OS software. The lower limit of quantification (LLOQ) and the limit of detection (LOD) were calculated for steroids and steroid levels were measured in plasma samples. The Formula Finder and ChemSpider were used to match MS/MS spectra to chemical structures. EAD-derived fragment ions were used to structurally characterize the steroids of interest. EAD generated structure-specific fragment ions that were sufficient to distinguish steroid isomers and isobars during analysis without requiring extensive chromatographic development.

The method leverages the speed and sensitivity of the ZenoTOF 7600 system to enable robust, high-throughput analysis of hormonal steroids in human plasma. Fragment ions generated by EAD provide structural details that allow characterization that cannot be achieved using data collected by CID. The simultaneous use of CID- and EAD-based fragmentation during quantitative analysis supports high-throughput analysis while improving analyte specificity.

Lipidomic profile of human nasal mucus

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Airways mucus is a complex hydrogel biopolymer layer located in the conducting airways (Bansil and Turner, 2006; Lieleg and Ribbeck, 2011) and it is the dominant mechanical host defense system of the human lung (Hill et al., 2022). Its main functions include lubrication of the epithelia, maintenance of a hydrated layer, conditioning of inhaled air, as well as acting as a barrier to airborne pathogens such as microbes and air pollution (Bansil & Turner, 2006; Lai et al., 2009d). In general, mucus is mainly composed of water (~95% w/w), mucins (~0.2 to 5.0% w/v), globular proteins (~0.5% w/v), salts (~0.5 to 1.0% w/w), lipids (1–2% w/w), DNA, cells, and cellular debris (Leal et al., 2017 and references therein). However, to date the chemical composition of human nasal mucus has not been adequately characterized.

In this study we will focus on the lipid composition of human nasal mucus, knowing their important role in rheological properties and their contribution to the lubricating and surface tension properties of the mucus layer (Bansil &Turner, 2018). The mucus sample was harvested from human primary epithelial nasal cultures grown at the air-liquid interface. The sample (N=1) is a pool from three different donors and was analyzed for fatty acids (FA) and for phospholipids, primarily phosphatidylcholines (PCs), phosphatidylethanolamines (PEs), and sphingomyelin (SM), using liquid chromatography coupled to mass spectrometry at the time-of-flight mode (LC-QTOF). Preliminary results show a clear predominance of SM (51%), followed by FA (26%), PEs (17%) and PCs (6%) of the total mass of 57 identified species. The unsaturated detected lipids were the dominant species (60%), compared with the saturated lipids (40%). More specifically, 91% of SM are unsaturated with predominance of SM(d18:1/16:0), all PEs are unsaturated with predominance of PE (16:0/18:1) with contribution of 28% of the total detected PEs. In the PCs group 86% are unsaturated with predominance of PC (16:0/18:1) with 15% of the total detected PCs species. The fatty acid group was mainly saturated with palmitic acid predominating (80%) and among the unsaturated fatty acids oleic acid had the highest contribution with 8% of all detected FAs.

Development of a comprehensive large scale lipidomic platform and its application to cardiometabolic disease research.

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Introduction: Human metabolism is complex, involving many interrelated and overlapping pathways. The use of advanced lipidomic techniques such as liquid chromatography with tandem mass spectrometry has enabled researchers to probe the relationships between disease states and lipid metabolism. However, due to the high variation among individuals, large sample sizes are typically required to ascertain true associations with biological or clinical outcomes. Here, we highlight our simplified and robust framework to enable the profiling of 1000's of samples, with low technical variance and minimum down-time. We further highlight critical areas that impact profiling large cohorts. Lastly, we feature lipidomic profiling of two large population studies and highlight the impact of larger population datasets in association studies.

Method: A targeted lipidomics platform was developed using reverse phase chromatography, utilising an Agilent 1290 Infinity II LC system with a ZORBAX Eclipse Plus C18, 100 × 2.1 mm, 1.8 μm HPLC column and water, acetonitrile and isopropanol solvent gradients (A, 50%:30%:20% v/v, B, 1%:9%,90% v/v respectively) with ammonium formate (10mM) and medronic acid (5uM in solvent A). This was used in conjunction with an Agilent 6495C QqQ mass spectrometer running in dynamic MRM mode and polarity switching. Complementary structural characterisation studies were run using a combination of high-resolution mass spectrometry (Thermo HFx), adduct modification (lithium acetate), synthesized standards, and chemical modification (acid hydrolysis). Quality control samples included the NIST SRM 1950 and pooled plasma samples run over the entire course of a large study.

Discussion / Results: Our lipidomics platform comprises a total of 763 lipid species spanning 44 lipid classes and subclasses, with a throughput of 16 minutes per sample (13 minutes in a dual column method). An average technical coefficient of variance of 8.6% (median 7.0%) was achieved for a sample set of 2,500 individuals. The characterised lipid list includes atypical lipid species characterised during development of the platform, including branched glycerophospholipids and modified sterol esters. We subsequently report their relationship with several cardiometabolic risk factors using two large cohort studies (the Busselton Health Study, n = 4,492 and the AusDiab study, n = 10,339) and highlight results from analytical approaches only possible with the larger datasets.

Method development for the analysis of short chain fatty acids by direct infusion mass spectrometry

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P38 — PRIZE

Objectives: Short chain fatty acids (SCFAs) are metabolites produced by the fermentation of complex carbohydrates by the gut microbiota. They play a pivotal role in people with multiple sclerosis (pwMS) and other neuroimmunologic diseases. Recent studies have shown that the serum and feces of pwMS contain significantly reduced amounts of propionate compared to controls. In this context, quantitative analysis of the SCFAs has the potential to improve our understanding of the mechanisms of the disease development and to aid in targeted studies on SCFAs supplementation. In this work, we present a simple, rapid, semi-automated, direct infusion mass spectrometry (DI-MS) approach for the quantitative analysis of SCFAs.

Methods: A new workflow has been developed for the analysis of SCFAs (acetate, propionate and butyrate) in serum, plasma and stool. It combines a specific protocol for extraction and preparation for MS analysis and a targeted DI-MS method for SCFAs. The latter includes determination of the lower limits of detection and linearity range for the three SCFAs. The developed method was further used for quantitative analysis of propionate in serum of healthy subjects before and after its oral supplementation.

Results: The semi-automated DI-MS approach substantially simplifies the SCFA quantification and allows a reduction in MS analysis time to be reduced to 2 minutes. The linear detection range of the DI-MS method is from 1 to 100 µg/mL for acetate (R^2=0.973), from 0.01 to 10 µg/mL for propionate (R^2=0.953), and 0.05 to 50 µg/mL for butyrate (R^2=0.993). Subsequent measurements of SCFAs in blood plasma, serum, and stool samples showed that the measured levels were within the expected range.

Conclusions: Our newly developed mass spectrometry-based workflow provides a significantly improved method for quantifying SCFAs. Due to its simplicity and shorter analysis time, it is particularly advantageous for use in clinical studies with many samples.

Lipidome remodeling during adaptation of Botrytis cinerea to growth at low temperature

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Cold storage is widely used during handling of fresh produce between production and marketing to extend shelf life and reduce losses. *B. cinerea* is a psychrotolerant fungus that can grow, infect, and cause fruit deterioration at low temperature (LT, near 0°C). In order to study the cellular adaptation of *B. cinerea* to LT, a time course experiment was performed during 72h after shifting the fungal culture from 22°C to 5°C.   Transcriptomic and Liquid chromatography-mass spectrometry (LC/MS) based lipidomics analyses indicated significant differences in gene expression and in lipid composition in response to the cold-shift. Genes related to biosynthesis of triacylglycerols (TAG) encoding for glycerol-3-phosphate acyltransferase and TAG lipases were significantly upregulated 2h after the shift and were followed by significant changes in TAG composition. Furthermore, during the first 4h of the cold stress, expression of genes encoding for sphingolipids (SL) hydroxylase, methyltransferase and desaturase was significantly altered, that was followed by a change in sphingoid bases composition. The change in membrane lipid composition was accompanied by expression of two distinct sets of genes encoding for membrane-bound hexose transporters, at 2h and 48h after the shift. These evidences suggest an important role for cellular modifications of the plasma membrane lipid composition in response to thermal variations to support proper functionality during adaptation to LT.

ASO-Mediated Inhibition of Degs1 Aggravates Inflammation and Hepatic Fibrosis in Mice by Increasing Free Cholesterol Storage

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Introduction
In a recent study[1], we demonstrated the connection between dihydroceramides and non-alcoholic fatty liver disease (NAFLD) progression. Additionally, we found that fenretinide, an inhibitor of dihydroceramide desaturase-1 (Degs1), the enzyme responsible for dihydroceramide conversion into ceramide, decreased steatosis in a mice model of non-alcoholic steatohepatitis (NASH). However, fenretinide may have off-target effects besides Degs1 inhibition. In this study, we explored the effects of an antisense oligonucleotide to specifically block Degs1 (ASO-Degs1) in a mice model of NAFLD.

Methods
Mice were fed a high-fat diet for up to 30 weeks. ASO-Degs1 was administered intraperitoneally for 8 weeks, and mice sacrificed at 14, 22, and 30 weeks to evaluate the effects on simple steatosis, steatohepatitis, and fibrosis. Lipidomics using RPLC-MS/MS was used to monitor the concentrations of sphingolipids, phospholipids, and neutral lipids in the liver and plasma of animals. The effects on grading and staging were evaluated histologically.

Results
Treatment with ASO-Degs1 reduced the RNA expression and protein levels of Degs1 which results in increased concentrations of dihydrosphingolipids (dhCer, dhSM and dhHexCer) in the liver and plasma of animals. Treated mice showed reduced adipose tissue accumulation and were leaner compared to controls. Histologically, they had less steatosis, which correlates with lower triglyceride content, but demonstrated a marked increase in the inflammatory activity and fibrotic damage. Additionally, livers were enlarged, showing increased concentrations of free cholesterol in whole liver, liver-isolated hepatocytes and macrophages. RNAseq expression analysis of liver revealed ASO-Degs1 mediated stimulation of cholesterol efflux pathways and inhibition in de novo cholesterol synthesis, in response to dihydrospingolipid accumulation, which is compatible with the significant increase in liver free cholesterol observed in plasma. On the other hand, single-cell RNAseq analysis provide a link between the increased expression of several markers (Spp1, Trem2, Lgals3), with an increase in the recruitment and activation of inflammatory cells from the monocyte/macrophage lineage.

Conclusion
ASO-mediated inhibition of Degs1 reduces steatosis but increases free cholesterol in the liver which triggers the activation of inflammatory cell pools, resulting in fibrosis.

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Metabolomic analysis of human plasma by bio-inert UHPLC system coupled with high-resolution mass spectrometry

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P41 **PRIZE**

Metabolomics is a comprehensive study, which is concerned with both nontargeted and targeted analysis of endogenous metabolites and attempts to systematically identify and quantify metabolites from a biological sample. Metabolites are small molecules typically up to 1,500 Da with diverse structures, including lipids, amino acids, peptides, nucleic acids, organic acids, vitamins, thiols, or carbohydrates. The metabolomic analysis is mainly based on mass spectrometry (MS) coupled with ultrahigh-performance liquid chromatography in HILIC or RP modes. The metabolites often contain problematic functional groups for the separation, like phosphate, which may interact with the surface of the instrument or chromatographic column, leading to bad peak shapes. The new instrumentation and columns with bio-inert materials offer excellent separation efficiency for these problematic analytes. The essential step of the metabolomic analysis is a sample preparation. Protein precipitation by organic solvents is the most commonly used method for sample preparation, but abundant lipid classes could be present in a large excess, which could cause the suppression of low abundant metabolites.

The bio-inert Acquity Premier UHPLC system with Acquity Premier BEH Amide column (150×2.1 mm; 1.7 µm) coupled to mass spectrometry with high-resolution (Xevo G2-XS QTOF, Waters) was used. The new separation method was developed with the following conditions: gradient elution, where the mobile phase A was acetonitrile with 0,005% acetic acid (AA), and phase B 15mM ammonium acetate with 0,005% AA, flow rate 0.4 mL/min, and column temperature 45°C. The total run time is 20 min, including the equilibration time. The MS conditions were optimized for both positive and negative ion modes. The method enables the separation of isomers, such as leucine/isoleucine/norleucine or determination of phosphate metabolites, such as ATP, ADP, and AMP. Double Folch extraction method was applied for the sample preparation, where the aqueous phase was collected for metabolomic analysis, and nonpolar lipid classes are eliminated to the organic phase. The optimized method was used for the identification of metabolites in human plasma based on the mass accuracy (less than 10 ppm). In total, we have identified more than 100 metabolites. including amino acids, saccharides, nucleotides, purine/pyrimidine conjugates, *etc*.

Lipid perturbation induced by Th2-type cytokines in 3D human epidermal equivalents: Prevention upon inhibition of the JAK/STAT signaling pathway.

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The immune response in atopic dermatitis (AD) is driven predominantly by T helper (Th2) cells. The Th2-type cytokines interleukin (IL)-4, and IL-13 activate the Janus kinase/signal transduction and activator of transcription (JAK-STAT) signaling pathway, which is essential for their pro-inflammatory action and detrimental effects on the permeability barrier in the epidermis. The lipid abnormalities driven by Th2-type cytokines have not been completely elucidated in AD. The objective of this study was to investigate the mechanisms of lipid derangement induced by Th2-type cytokines in the 3D-epidermal equivalent model of AD. The role played by JAK/STAT signaling pathway lipid perturbations induced by Th2 cytokines was delineated by using the JAK inhibitor tofacitinib. Th2-type cytokines caused decrement in the elongation process of fatty acids and in the ceramide synthesis, as demonstrated by the reduced levels of ELOVLs 1, 3, and 6, and SPT mRNAs. In contrast, mRNAs of the lipid metabolism-related enzymes DEGS2 and CA2 were increased. Expression levels of PPARG mRNA were also significantly decreased. Altogether, these effects were abrogated by tofacitinib. Th2 signals caused a significant depletion of triglycerides (TGs) paralleled by a lower content of intracellular lipid droplets and increased levels of phosphatidylcholines (PCs). The depletion of TGs was partly associated with the inhibition of the monounsaturated palmitoleic acid formation. Likely, the observed lipid abnormalities occurred prior to changes in ceramide profiles, which were minimally perturbed. Lipid changes induced by Th2-type cytokines were abrogated upon co-treatment with tofacitinib. In conclusion, inhibition of JAK/STAT activation effectively abrogates the derangement of the lipid metabolism mediated by Th2-type cytokines.

Understanding the role of plasmalogen lipid in synaptic function: a cellular neurolipidomics study

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Lipids make up half the dry weight of the human brain where they support critical functions in nerve cells. Yet neuroscientists pay little attention to them. The scope of 'neurolipidomics' is to discover how lipid changes affect neuronal and glial cell function to better understand the physiology and pathology of the nervous system. In our laboratory, to unveil brain lipid function, we determine lipid levels while we study lipid interacting partners in neural cells. By integrating MS-based lipid quantification with analyses of lipid-related enzymes, we attempt to unveil their specificity, localization, and regulation. Also, we are puzzled by lipid shapes and physical properties, and inspired by biophysical studies we seek to probe lipid–lipid and lipid–protein interactions in neural membranes. Finally, we attempt to understand the relationship between the neural lipidome and its partners with an integrated outlook. Our focus is on how lipids alter the geometric properties and supramolecular organization of neuronal and glial membranes.

Plasmalogens are a type of phospholipid that is abundant in the brain, both in grey and white matter. Here, ethanolamine plasmalogens (PE-P) are abundant in the plasma membrane while choline plasmalogens are confined to mitochondria. Specifically at neuronal synapses, PE-P is enriched with PUFA, which together with the characteristic vinyl-ether bond and the narrow ethanolamine polar head confer to the lipid a pronounced conical shape. This is ideal to support vesicle release by reducing the energy required for vesicle fusion at the plasma membrane. In line with relevant findings in a plasmalogen-depleted mouse - displaying impaired neurotransmission - it has been speculated that plasmalogen may support the firing of signals along nerve fibres. Furthermore, plasmalogen levels reduce with age and are linked to neurodegenerative diseases, including Alzheimer's. Here, plasmalogen loss occurs early, perhaps contributing to the pathological progression ultimately leading to dysfunctions in memory, sensation, and cognition.

In the present study, we seek to provide direct evidence of the function of synaptic plasmalogen, and we use SH-SY5Y human neuroblastoma cells to build a simple but informative model of neurons. Here we measure synaptic assembly and in parallel monitor lipid levels by ESI-HRMS/MS shotgun lipidomics. To prove our hypothesis that plasmalogen homeostasis is critical for synapse function we use a combination of synaptogenic astrocyte-conditioned media, depletion of neuronal plasmalogen content by gene silencing, and replenishment by supplementation and then compare neurons in different conditions with downstream readouts.

Our preliminary data show that neurons respond to synaptogenic media by increasing simultaneously both plasmalogen levels and synaptic assembly. Experiments on plasmalogen depletion and supplementation are on the way and we are introducing functional electrophysiological measurements. By translating to relevant iPSC-based disease models, our approach could be used to study plasmalogen-replacement therapy as a treatment for neurodegeneration.

Discerning the functional role of plasmalogen at the synapse by shotgun lipidomics

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P44 — PRIZE

Phosphatidylethanolamine plasmalogen (PE-P) is a phospholipid subclass enriched in the brain. In grey matter – where neurons are most abundant – PE-P is enriched with PUFA; whilst in the myelinated white matter, PE-P is usually esterified with MUFA at the sn-2 position. PE-P possess unique physicochemical properties owing to their characteristic vinyl ether bond. This bond narrows the cross-sectional area of the polar head, conferring the lipid a conical shape and propensity for the inverted-hexagonal phase, making them specialised in easing negative membrane curvature and facilitating membrane fusion events. Furthermore, the chemical lability of the vinyl-ether bond enables plasmalogens to scavenge ROS and protect nerve cells from oxidative stress.

The non-lamellar biophysical properties would be valuable within the brain, both in the highly curved myelin that wraps around neuronal axons, and at synapses where efficient vesiculation is crucial for neuronal function. In addition, the sensitivity to oxidation can explain why plasmalogen decreases in normal ageing and in several neurodegenerative diseases featuring unmitigated oxidative stress.

For example, in Alzheimer's disease, synaptic dysfunction develops early driving the gradual impairment of memory, sensation, and cognition. This dysfunction correlates with a decline in levels of PE-P in grey matter, however, direct evidence of the functional role of PE-P at synapses is still missing.

To fill this gap, we developed a simple in vitro model of neurons to assay synapse function while modulating levels of PE-P. SH-SY5Y neuroblastoma cells are differentiated into neuron-like cells by serum starvation and addition of neurotropic factors to make them post-mitotic and polarised, prompting formation of an extensive neural network. Neurons are incubated with synaptogenic astrocyte-conditioned media, and plasmalogen levels are modulated by i) post-transcriptional silencing of plasmalogen biosynthetic enzyme FAR1 and/or ii) plasmalogen supplementation . Downstream analyses include PE and PE-P quantitation by shotgun lipidomics in conjunction with assays of protein expression via Western blot and synaptic assembly via immunofluorescence microscopy.

For targeted ESI-HRMS/MS shotgun lipidomics, an LTQ-Orbitrap XL mass spectrometer (Thermo Fisher Scientific) is coupled with a Triversa NanoMate (Advion Biosciences). Lipids of interest are identified by product-ion scan MSn analyses. We target this approach to identify and quantify PE-P, PE-O and PE species, with aims to expand into global lipidomics to detect compensatory mechanisms.

Our preliminary data indicates that plasmalogen level and composition influence synapse assembly, with further experiments being implemented to determine underlying mechanisms. Our research could provide novel insights into the function of PE-P at synapses and the potential of plasmalogen supplementation for treating synapse dysfunction in neurodegenerative disorders.

Hepatic lipid profiling - Deciphering hepatic lipid metabolism with time and species resolution

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P45 PRIZE

Fatty liver, liver failures, impaired regeneration capacity of liver and alcoholic liver diseases are all also caused as a result of distorted hepatic lipid composition. Phosphatidylcholines are the most abundant phospholipids present. PC syntheses are carried out by Kennedy pathway and lands cycle pathway (remodelling). In liver, the PEMT pathway also plays a key role in these dynamics. We are aware about the different pathways, but much is yet to be unravelled about these pathways, their kinetics, and the different species (chain lengths and unsaturation) formed and whether they have any physiological applications or might result in any malfunctions. Metabolic pathways are overly complex and interlinked. Studying each individual pathways will help us identify and understand the functions. Liver, the hub of lipid metabolism, helps us understand the overall lipid turnover and status of the body, thus primary hepatocytes form the most promising model to study. Here we are using primary hepatocytes from murine model for our experiments. A combination of alkyne and/or isotope labelled fatty acids, click chemistry and pathway inhibitors together with the resolving power of Mass spectrometry (direct infusion) are used to facilitate this study. On the long run we would also like to comprehend the effects each pathway and the different lipid species have on these diseases.

QUANTITATIVE ANALYSIS OF THE STREPTOCOCCUS PNEUMONIAE LIPIDOME

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P46 **PRIZE**

The membrane composition plays a crucial role in the structure and protection of bacterial cells. The understanding of the relationship between genetic inventory, biosynthesis, and environment on one side and the composition of microbial lipidomes on the other is still limited. Specifically, for *Streptococcus pneumoniae* (*Spn*) only qualitative data is available. In our study, we employed shotgun lipidomics to investigate the impact of selected genetic mutations (Δ*tacL*, Δ*lgt*, and Δ*cps*) on the membrane lipid composition of *Spn* strain D39. A customized mix of internal standards enabled reliable quantitative analysis. Our analysis led to the quantification of more than 100 lipids of six classes (DAG, GlcDAG, GalGlcDAG, PG, CL and PC) with GlcDAG and GalGlcDAG being the major abundant classes. Only very minute amounts of lysolipids (LPG, LPE) were detected in our study. Fatty acid profiling by Gas Chromatography/Mass Spectrometry and Thin Layer Chromatography combined with high resolution MS^2 were further used for identification of lipid species. Fatty acid profiling showed that palmitic, stearic, and oleic acid are the predominant fatty acids. The lipidome of Δ*tacL* and Δ*lgt* strains showed no significant changes compared to the wild type, while the nonencapsulated strain exhibited significant adaptation in lipid composition.

In summary, we have established a robust method for quantitative lipidome analysis in *Spn*, enabling future investigations of other mutants or growth conditions to shed light on membrane homeostasis and biosynthesis regulation.

Longitudinal analysis of maternal plasma and urine L-carnitine and acylcarnitine species in association with metabolic risk factors

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Maternal plasma levels of carnitine and acylcarnitines decrease as the pregnancy progresses that may lead to secondary carnitine deficiency with implications on maternal health and child development. The maternal circulating carnitine levels can be influenced by diet, maternal-fetal transfer as well as pregnancy-induced increase in excretion, however, the underlying mechanism is not clearly understood. We carried out a concurrent analysis of maternal carnitine and acylcarnitines in both plasma and urine from a preconception and longitudinal cohort (n=804). We observed decreased plasma concentration and increased carnitine excretion in pregnancy. At three months postpartum, plasma carnitine levels and carnitine excretion reverted to preconception levels. Short-chain acylcarnitines were positively associated with pre-pregnancy BMI (ppBMI) at the three physiological states. Medium and long-chain acylcarnitines were negatively associated with ppBMI in two non-pregnant states whereas these were positively associated with ppBMI during pregnancy. Gestational weight gain (GWG) was positively associated with short-chain acylcarnitines, but negatively associated with medium- and long-chain acylcarnitines during pregnancy. Urine acylcarnitines in pregnancy were positively associated with ppBMI and GWG. Using urine-to-plasma ratios as an indicator of carnitine excretion, we observed an increase in carnitine excretion in pregnancy with increasing ppBMI. Similarly, carnitine excretion in pregnancy was higher in mothers with higher GWG. Plasma short-chain acylcarnitines were positively associated with glycaemic traits at the three states except for acetylcarnitine (C2). Plasma medium and long-chain acylcarnitines were negatively associated with fasting insulin and HOMA-IR at preconception and pregnancy. Association analysis of plasma and urine carnitine and acylcarnitines identified preconception propionylcarnitine (C3) concentration to be positively associated with gestational diabetes (GDM), suggesting its potential as an early detection marker for GDM at preconception. Further, GDM mothers had increased concentrations of hexanoylcarnitine (C6) and hexenoylcarnitine (C6:1) in pregnancy. GDM mothers had lower concentrations of medium and long-chain acylcarnitines at postpartum compared to mothers with normal glucose metabolism. Overall, we provide a detailed overview of maternal circulating and excretory levels of carnitine from preconception through pregnancy to postpartum. Our analyses provide in-depth insights into a pregnancy-induced reduction in circulating carnitine levels and the potential utility of plasma/urine carnitine and acylcarnitines in the assessment of maternal cardiometabolic risk.

Decoding the complex composition of exossomal lipids

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Extracellular vesicles (EVs) are small structures secreted by eukaryotic cells that are capable of exerting profound impacts on biological processes. Hence, they are a crucial target for biomarker research. Although small, the makeup of EVs is multifaceted, including lipids, genetic material, proteins, and metabolites. The high heterogeneity and small concentrations of EV samples pose an analytical challenge. As membranous structures, lipids are involved in EV maturation, secretion, and function. The characterization of the EV lipid composition could help us better define the purpose of these structures, as well as further advance biomarker research.

In this study, we employed nanoLC and UHPLC-MS to explore the lipid composition of EVs isolated from serum and cell samples. Firstly, we utilized a high-sensitivity nanoLC-MS method to analyze the composition of serum exosomes (small endosomal EVs) against the source samples. NanoLC is an ideal technique to analyze small amounts of diluted biological samples due to the extremely high sensitivities, but the low robustness usually associated with most methodologies must be carefully assessed. Upon comparing the EVs with the parent serum, our results revealed a decrease of glycerophospholipids and ceramides levels and heightening of lipids active in membrane fusion and invagination. We will further discuss the biological implications of our findings and the merits of the employed method. Secondly, we employed a comprehensive UHPLC-MS platform to study the lipid composition of EVs from cell lines. We optimized a methodology that includes sample preparation, analysis, and data processing for high-sensitivity UHPLC-MS lipidomics that combines extensive lipid coverage with improved sample throughput and robustness. We identified over 2000 lipids, including high proportions of phosphatidic acids and glycerophosphoserines. We also detected triacylglycerols, cholesteryl esters and sterols. Although these species are not usually reported in EVs, we have identified them in multiple studies when applying high-sensitivity methods, underlining the importance of advanced platforms for specialized applications. Thirdly, we examined how synthetic RNA transfection complexes affect the composition of EVs. Lipid-based transfection complexes are often employed as drug delivery systems to cells. Once absorbed, the complexes are processed by endosomes, organelles that may eventually maturate to become EVs. Our findings indicated the presence of lipids from the transfection complex in EVs isolated from transfected cells after thoroughly washing, which indicates contamination of EV preparations. Hence, the conclusions of studies regarding the function of EVs from transfected cells must be carefully evaluated. We will delve into the impact of our results on biochemical processes and highlight the benefits and limitations of our techniques.

New structural insights for gangliosides using high-resolution mass spectrometry with Ultraviolet Photodissociation and Electron-activated dissociation fragmentation strategies

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Gangliosides are important glycolipids present in mammalian cells. They are most prominent in the nervous system with important functions related to cell-cell communication, cellular growth, host-pathogen interaction, and signal transduction. Ganglioside analysis is challenged by their chemical complexity involving a glycan and lipid part as well as through limited commercial standards and annotation strategies. High-resolution mass spectrometry (HRMS) enables the structural analysis of gangliosides up to the molecular lipid species level.[1] State-of-the-art HRMS workflows involve thermal fragmentation strategies such as collision-induced dissociation (CID) or high-collision dissociation (HCD). In this work, we investigate novel radical-driven fragmentation strategies for their value in gaining additional structural information of gangliosides. Analyzing commercially available ganglioside standards as well as a selected panel of samples, we investigate the fragmentation of (1) Ultraviolet Photodissociation (UVPD) on a Orbitrap Fusion™ Lumos™ Tribrid™ Mass Spectrometer (Thermo Scientific) using a 193 nm laser and (2) Electron Activated Dissociation strategies on a ZenoTOF 7600 System (AB Sciex). Data analysis is performed using the open-source Lipid Data Analyzer (LDA) for automated ganglioside annotation. So far, we identified significantly more fragments comparing UVPD and EAD-based approaches with state-of-the-art CID-based gangliosides fragmentation. In our opinion, novel fragmentation strategies in combination with automated annotation will significantly improve glycolipid analysis workflows.

1. Hohenwallner, K. *et al.* Decoding Distinct Ganglioside Patterns of Native and Differentiated Mesenchymal Stem Cells by a Novel Glycolipidomics Profiling Strategy. (2022) doi:10.1021/jacsau.2c00230.

Metachromatic leukodystrophy diagnosis in Morocco: exploration of sulfatides diversity in urine samples of infantile patients using molecular networks

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P50

Sulfatides (3-O-sulfogalactosylceramides) are major lipidic components of the myelin sheath of neurons. Alterations in sulfatide metabolism are associated with several human diseases [1]. In particular, metachromatic leukodystrophy (MLD) is a lysosomal storage disease characterized by an enzymatic dysfunction of Arylsulfatase A (ARSA), leading to an intralysosomal over-accumulation of sulfatides. MLD patients suffer from progressive motor and cognitive impairments, with no current effective treatment [2]. As urinary sulfatides are gold biomarkers to diagnose and monitor therapy efficiency [3-4], our goal was to improve the existing analytical methods, using molecular networks.

Lipid extracts obtained from urine samples of a normal subject and 6 MLD patients (from 2 to 3 years of age) were analyzed through UHPLC-HRMS profiling experiments performed with an Orbitrap Q-Exactive Focus system. The resulting data were processed using the Feature-Based Molecular Networking (FBMN) workflow in the Global Natural Products Social Networking (GNPS) platform, with automatic annotation proposed by the GNPS tool and manual annotation based on the comparison with an analytical standard and literature data.

A large diversity sulfatides was observed in the samples, with saturated and unsaturated C16 to C24 fatty acyl chains, including some hydroxylated species. Interestingly, the molecular network aggregated sulfolactosylceramides alongside with the sulfatides, the sulfatides precursors galactosylceramides, and one ceramide. As sulfatides accumulation in neurons was shown to contribute to disease phenotype in a mouse model [5], exploring the chemodiversity of sulfolipids in MLD patients' urine samples could help to progress in the understanding of the physiological mechanisms of this pathology.

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Novel lipid biomarkers for algal resistance to viral infection in the ocean

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Algal blooms, rapid proliferation events of marine microalgae, are of great importance to the marine food web and to the biogeochemical cycles of nutrients, such as carbon and sulfur. The extent and duration of these blooms are controlled by multiple environmental factors, including viruses. Viruses are the most abundant biological entity in the marine environment and serve as major evolutionary and biogeochemical drivers in the ocean. Algae-infecting viruses are estimated to turn over more than a quarter of the photosynthetically fixed carbon. Tapping into the arms-race between an algal host and its virus can reveal unique strategies of viral infectivity and host defence.

An ecologically important marine host-virus model system is the cosmopolitan alga *Emiliania huxleyi* and its specific virus, *E. huxleyi* Virus (EhV), a large dsDNA virus. *E. huxleyi* forms massive blooms that cover vast oceanic areas and are frequently terminated due to infection by EhV. EhV infection causes profound remodelling of the *E. huxleyi* lipidome, including induction of virus-derived glycosphingolipid (vGSL) biosynthesis, which can lead to host cell death. Resistance to viral infection has been reported for various algal species under laboratory conditions, including some strains of *E. huxleyi*. Nevertheless, the occurrence of resistant cells in natural populations is underexplored due to the lack of sensitive tools to detect these rare phenotypes. Consequently, our current understanding of the ecological importance of resistance and its underlying mechanisms is limited.

In this study, we sought to discover lipid biomarkers for the resistance of *E. huxleyi* to its specific virus. We applied an untargeted LC-MS-based lipidomics approach and identified a novel group of glycosphingolipid (GSL) biomarkers that characterize resistant *E. huxleyi* strains, varying in their level of long chain base hydroxylation and saturation compared to the host GSL species. We detected these biomarkers in open ocean *E. huxleyi* blooms and in isolates collected from a small scale bloom, indicating that resistant cells predominantly occur during the demise phase of the bloom. Lastly, we show that the GSL composition of *E. huxleyi* cultures that recover following infection and gain resistance to the virus resembles that of resistant strains. These findings highlight the metabolic plasticity and co-evolution of the GSL biosynthetic pathway and underscore its central part in this host-virus arms race. This work has now been accepted to PNAS.

Application of ion mobility - tandem mass spectrometry in the analysis of isobars and structural isomers of bacterial lipids

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Glycosylglycerolipids, together with phospholipids, are essential components of bacterial membranes. These lipids exert central roles in physiological processes such as maintaining membrane stability. Lipopolysaccharides are abundantly present in the outer cell envelope of Gram-negative bacteria, with lipid A as its membrane anchoring component that is associated with bacterial toxicity. Moreover, modification of lipid A plays a central role in bacterial resistance against antibiotics, i.e. against the last-line reserve antibiotic colistin.[1,2] Such molecules frequently occur as structural isomers, that are not differentiable by MS[1] measurements due to identical monoisotopic masses. Even MS[2] analyses not always lead to sufficient structural information because of the lack of indicative fragments.

Here, we present first data on the potential of cyclic IMS-MS^n adding an additional analytic dimension for different challenges we faced during our research on bacterial cell wall glycolipids: 1) presence of overlapping ion clusters in the shotgun analysis of the pneumococcal lipidome, 2) presence of two mono-hexosylglycerolipids with different sugar moiety in the glycolipid extract of *Streptococcus suis*, 3) presence of structural isomers in lipid A preparations, i.e. of *Pseudomonas syringe*[3] and 4) the need of discrimination of PI 35:0−16:0_19:0 (TSA) derived from *Mycobacterium tuberculosis* from PI 35:0-species being present in the flora of healthy human subjects[4]. Examples will be presented of how IMS-MS^n can support in sorting out such challenges.

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Data Independent Acquisition for the Dissection of the Unfolded Protein Response in Glioma

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P53   PRIZE

Glioblastoma is a highly malignant brain tumor derived from glial cells, and it poses a significant therapeutic challenge due to its aggressive nature and high resistance to treatment. The unfolded protein response (UPR) has emerged as a promising therapeutic target for glioblastoma due to its multifaceted role in regulating cellular processes beyond protein folding stress, including inflammation, lipid metabolism, energy homeostasis, and cell differentiation[1,2].

The UPR comprises three branches, IRE1α, ATF6, and PERK, which collaborate to mitigate endoplasmic reticulum (ER) stress and restore ER homeostasis[3,4]. To investigate the UPR's potential as a therapeutic target for glioblastoma, we employed a DIA proteomics approach to monitor protein expression changes following UPR induction and UPR-branch-specific inhibition in NHA and NCH421K cell lines.

Our findings revealed branch-specific regulation of the UPR, and clustering analysis uncovered the PERK pathway's pivotal role in regulating lipid metabolism-related genes under stress conditions. Future studies incorporating additional assays and lipidomics analysis will further validate these novel findings.

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Multidimensional lipidomic reveals specific adipose-derived extracellular vesicle lipid sorting informative of the obesity metabolic state

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Adipose extracellular vesicles (AdEVs) transport lipids that could participate in the development of obesity related metabolic dysfunctions. We used multidimensional lipidomics to define mouse AdEV lipid signature in either healthy or obesity context. Based on HILIC and C18 liquid chromatography separation coupled to tandem mass spectrometry (MS/MS) analysis, we were able to detect up to 313 lipid species from 15 different lipid subclasses among which neutral lipids (TG, DG, CE, FC), glycerophospholipids (PC, PE, PI, PG, PS, PA, LPC, LPE) and sphingolipids (SM, dihydroceramides and ceramides) in three different matrices (plasma, extracellular vesicles and adipose tissue). Distinct clustering of AdEV and visceral adipose tissue (VAT) lipidomes by principal component analysis reveals specific AdEV lipid sorting when compared with secreting VAT. Comprehensive analysis identifies enrichment of ceramides, sphingomyelins and phosphatidylglycerol species in AdEVs compared with source VAT whose lipid content closely relates to the obesity status and is influenced by the diet. Obesity moreover impacts AdEV lipidome, mirroring lipid alterations retrieved in plasma and VAT. Overall, our study identifies specific lipid fingerprints for plasma, VAT, and AdEVs that are informative of the metabolic status. Lipid species enriched in AdEVs in the obesity context may constitute biomarker candidates or mediators of the obesity-associated metabolic dysfunctions.

Lipidome analysis of HIV-infected cell line models

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As of today, more than 38 million people around the world are persistently infected with the human immunodeficiency virus (HIV). Effective anti-retroviral therapy (ART) is available that stops viral replication and disease progression. However, the virus is able to persist in latently infected cells, despite ART, and elimination or even identification of these so-called viral reservoirs is virtually impossible. A reactivation of latent HIV for subsequent eradication of the infected cell might be a curative strategy. Latency reversal agents (LRA) potently inducing viral transcription in cell culture have been described but in clinical studies, the reversal of latency by these molecules was not satisfying or resulted in severe side effects.

It has been shown that an increased cholesterol efflux impedes the disease progression and that statin treatment might reduce the risk of a failure of ART. Thus, hypolipodemic drugs could affect viral persistence.

Here, we have used a cell line model of latency, called J-Lat cells to investigate the role of the lipid metabolism in HIV persistence. These T cells either solely harbor a GFP reporter gene under the control of the HIV promotor or additionally the key HIV transcriptional regulator tat. In addition, we investigated the lipid composition of cells infected with a dual-reporter virus that allows a separation of productively and latently infected, as well as uninfected control cells by flow cytometry. HPTLC and by HPTLC coupled to mass spectrometry were then used to investigate the lipid composition of these cells.

The results of this very first lipidome investigation of HIV-infected cells show clear differences within the lipidome of the different infection states. The most pronounced differences were found for the lipid classes of ether-phosphatidylethanolamines, sphingomyelins and apolar lipids. These differences might account for a change in the rigidity and the stiffness of the cellular membrane, which appears to have hitherto unknown effects on HIV transcription. Our findings could have important implications for the development of novel diagnostics and therapeutic strategies for HIV.

Characterization of Diabetic Murine Extracellular Vesicle Lipids

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Many studies have linked Extracellular Vesicles (EVs) to disease progression. For example, EVs from diabetic and obese mice can induce insulin resistance while EVs from healthy mice can reverse these effects. EVs are lipid bilayer nanoparticles released from cells by a variety of mechanisms. These particles are known to contain a variety of cargo, such as lipids, proteins, nucleic acids, and other metabolites that are believed to be responsible for their cell signaling activity. For diabetes patients, islet transplantation has been shown to be a low risk and potentially effective alternative to organ transplant. There is evidence that EVs may improve islet transplantation, possibly through signaling between b-cells and EVs from endothelial cells. Characterizing lipids present in EVs is one piece of the puzzle in understanding the role they play in diseases such as diabetes. In this study we have performed lipidomics analysis on isolated plasma EVs from healthy and diabetic mice in an attempt to identify differences in their lipid profiles.

Changes in the mitochondrial phospholipidome of OPA1 and mitofusins knockouts of mouse embryonic fibroblasts: an investigation by hydrophilic interaction liquid chromatography with high-resolution mass spectrometry

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Beyond their crucial role in energy production through oxidative phosphorylation, mitochondria are currently recognized as the crossroad of numerous metabolic and signalling pathways displaying many interactions with other cytoplasmic structures. Thus, the study of mitochondrial disorders has progressively expanded to include those medical conditions that are related to defective mitochondrial dynamics and interactions with lysosomes and endoplasmic reticulum (ER) [1,2]. Nowadays, understanding how mitochondrial DNA and nuclear DNA defects leading to mitochondrial diseases (*e.g.*, dominant optic atrophy) impinge on the mitochondrial membranes' lipid profile still represents a largely unexplored landscape. In this study, the hyphenation of hydrophilic interaction liquid chromatography (HILIC) and high-resolution Fourier-transform mass spectrometry (FTMS) was exploited to make a further step towards the understanding of the complex relationship between the depletion of proteins involved in mitochondrial fusion and the lipid composition of mitochondrial membranes. The effects of two genetic modifications on the lipid composition of mitochondria in mouse embryonic fibroblasts (MEF) were examined. The first modification involved the inactivation of the optic atrophy 1 gene (OPA1 -/-), while the second modification involved the inactivation of mitofusin 1 and 2 genes (Mfn 1/2 -/-). The abundance ratios of key lipid classes (namely, phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI), and cardiolipin (CL)) in the isolated mitochondria were evaluated. The results showed that the abundance ratios of these lipid classes were not significantly changed in OPA1 -/- and Mfn 1/2 -/- mitochondria compared to wild-type MEF. However, there was a significant decrease in the relative occurrence of certain lipid species (alkylic/acylic species) in PC and PE in Mfn 1/2 -/- mitochondria and in PE in OPA1 -/- mitochondria. Moreover, lipid extracts from OPA1 -/- mitochondria showed a higher abundance of PC and PE species with highly unsaturated side chains. Regarding PI and CL, both types of knockouts displayed a higher presence of species with a greater ratio of double bonds (C=C) to the total number of carbon atoms in their side chains when compared to wild-type MEF. These findings suggest that the removal of OPA1 and Mfn 1/2 proteins not only disrupts fusion processes but also induces subtle changes in the lipid composition of MEF mitochondria, potentially affecting their ultrastructure.

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Mapping of the Human Cell Metabolome and Lipidome Identifies Novel Cancer Therapeutic Targets in MYC-amplified Group 3 Medulloblastoma

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Significant efforts have recently focused towards understanding metabolic reprogramming in cancer, attempting to discern context-specific biology exploitable for diagnosis or treatment. However, the coverage of metabolomics and lipidomics data is lacking relative to the transcriptome and proteome, which have been deeply profiled across different tissues and cell types. Hence, our rudimentary understanding of the human metabolome and lipidome is a substantial bottleneck towards discoveries. To improve our understanding of metabolic and lipidomic heterogeneity within cancer, we performed a deep mapping of the metabolome and lipidome of 25 human cell types from various tissues and created the Human Cell Metabolome Atlas (HCMA), containing relative quantification of ~1,000 endogenous metabolites and lipids. The HCMA yielded valuable insights into metabolites and lipids that are highly specific to individual types of cells and tissues including fatty acid specificity in distinct lipid classes. More importantly, our atlas provides a reference and the necessary context to determine the specificity of metabolic and lipidomic signatures in different cancer cell types. Here, I will present how these unique signatures can identify potential metabolic vulnerabilities using Group 3 Medulloblastoma (G3MB) – an aggressive MYC-amplified pediatric brain tumor characterized by therapy resistance, recurrence, and poor survival.

Pathway analysis of G3MB with cell lines in the HCMA indicate pyrimidine (https://doi.org/10.1016/j.ccell.2022.10.009) and cholesterol esters (CE) are uniquely regulated in G3MB. For example, fatty acid composition analysis showed a high specificity for ultra-long chain fatty acids (C >26) in CE, which we then validated in vivo. Further functional investigation using both genetic, and pharmacological inhibition of this pathway selectively targeted G3MB cell viability without impairing neural stem cells (the proposed cell of origin of G3MB) viability, as the HCMA predicted. Finally, we validated our results in vivo using PDX models.

Together, we anticipate further discoveries as our HCMA continues to grow, including novel diagnostic biomarkers and context-specific metabolic and lipidomic vulnerabilities within defined cancer lineages.

Exploring phosphatidyl-myo-inositol mannosides of Mycobacterium tuberculosis in different matrices by shotgun lipidomics

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P59

Tuberculosis (TB), one of the deadliest infectious diseases alongside malaria and HIV, caused 10.6 million new infections and 1.5 million deaths in 2022 according to the WHO. The predominant causative agent of TB, *Mycobacterium tuberculosis* (*Mtb*), is a rod-shaped bacterium that possesses a characteristic, waxy cell wall, in which various lipids are embedded. They play a crucial role in the pathogenicity and resistance of the bacteria against the immune system of the host.

Phosphatidyl-*myo*-inositol mannosides (PIMs) are besides phosphatidylinositol (PI), phosphatidylethanolamine, phosphatidylglycerol and cardiolipin the major phospholipid components of the *Mtb* plasma membrane (PM). PIMs are highly heterogeneous in structure due to their number of mannoses and acyl groups and can occur in di- to tetra-acylated forms. The most common species, found in *Mtb* includes phosphatidyl-*myo*-inositol dimannoside (PIM_2) and phosphatidyl-*myo*-inositol hexamannoside (PIM_6) and their respective tri- and tetra-acylated versions (Ac_1/Ac_2PIM_2 and Ac_1/Ac_2PIM_6). PIMs not only serve as intermediates in the synthesis of lipomannan and lipoarabinomannan, but specific molecular species also reside as metabolic end products in the inner and outer leaflet to contribute to the integrity and stability of the PM.[1] Due to their global presence in bacteria of the pathogenic *Mycobacterium tuberculosis* complex (MTBC), PIMs represent an interesting candidate to function as a lipid-based marker to detect infection as recently described for TSA-containing PIs.[2]

Hence, in this study, especially PIM_2 and its acylated forms are evaluated on their potential to serve as suitable marker to detect *Mtb* infections by applying a shotgun lipidomics methodology. The development of the workflow, starting from a whole lipid extract derived from the laboratory strain *Mycobacterium tuberculosis* H37Rv, will be presented.

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Single Cell Lipidomics: automated cell collection with ZenoTOF analysis

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P60

Single cell analysis is becoming an essential bioanalytic tool in unravelling the significance of cell-to-cell heterogeneity as previous research in cancer has demonstrated that methods for bulk cell or tissue analysis are not sensitive enough to reflect cell phenotypes only present in the minority of a mix of cells. To date, RNA- or DNA-based techniques, transcriptomics and genomics, have been used to characterise single cells, however, biological phenotyping on a single cell level may reveal novel insights into cellular functions via the comprehensive analysis of proteins, metabolites and specifically lipids (lipidomics).

Recent advances in sampling and analytical platforms have revolutionised our ability to perform omics of individual cells, here we demonstrate the success and challenges for lipidomics analysis of single cells based on novel automated cell selection using the Yokogawa SS2000, microflow RP18 chromatography and MS^2 based lipid identification using a ZenoTOF 7600.

Lipidomic Analysis Reveals Differences in Bacteroides Species Driven Largely by Plasmalogens, Glycerophosphoinositols and Certain Sphingolipids

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There has been increasing interest in bacterial lipids in recent years due, in part, to their emerging role as molecular signalling molecules. *Bacteroides thetaiotaomicron* is an important member of the mammalian gut microbiota that has been shown to produce sphingolipids (SP) that pass through the gut epithelial barrier to impact host SP metabolism and signal into host inflammation pathways. *B. thetaiotaomicron* also produces a novel family of *N*-acyl amines (called glycine lipids) that are potent ligands of host Toll-like receptor 2 (TLR2). In the present study, we specifically examine the lipid signatures of four species of gut-associated *Bacteroides*. In total we identify 170 different lipids, and we report that the range and diversity of *Bacteroides* lipids is species specific. Multivariate analysis reveals that the differences in the lipid signatures are largely driven by the presence and absence of plasmalogens, glycerophosphoinositols and certain SP. Moreover, we show that, in *B. thetaiotaomicron*, mutations altering either SP or glycine lipid biosynthesis result in significant changes in the levels of other lipids, suggesting the existence of a compensatory mechanisms required to maintain the functionality of the bacterial membrane.

Navigating the Lipid Universe with LipidLibrarian: The Ultimate Search Engine for Lipids

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There are many public resources and guidelines available for lipidomics research, like standard nomenclatures, classification systems, and databases for lipids. However, these resources and guidelines are not always aligned with one another, meaning that databases use different classification systems and nomenclature styles, making it difficult to find the same lipid across different databases. These databases contain many common attributes on each lipid, but provide unique data on their respective speciality. As of now, the standard workflow to acquire lipid information is to query all the available public databases individually, then tediously compare and aggregate the results manually. This workflow is not feasible with the vast amounts of data generated via high throughput mass spectrometry. Furthermore, it represents another barrier to entry into the subject area of lipidomics for beginners and students.

To tackle these challenges we present LipidLibrarian, a lipid search engine that enables a combined search of all big lipid databases by aggregating the available information and presenting it in a unified manner. The three pillars that make LipidLibrarian into a well-rounded and extensive search-engine are SwissLipids, LIPID MAPS and Alex123. LIPID MAPS has a large ecosystem of utilites which can be used in a variety of lipid related research use cases. SwissLipids contains the Rhea reactions of a lipid and Alex123 is the only database containing lipid fragment information. Additionally we enriched the results with data from LION, and Rhea and Reactome through LINEX. To perform name and hierarchy conversions, we use LipidLynxX and Goslin. LipidLibrarian is developed as an open-source application with a focus on extensibility to easily integrate new resources.

LipidLibrarian is accessible via a user-friendly website allowing the user to query lipids using their trivial names, shorthand notations, database identifiers, or their masses. Alternatively, LipidLibrarian can be accessed as a Python package, which can be integrated into high-throughput lipidomics pipelines. The software is intended for a wide range of users, covering beginners to experts in molecular biology, computational biology, and lipidomics research.

The output of LipidLibrarian is presented in an intuitive results page, consisting of multiple categories that hold information on the queried lipid: nomenclature, database identifiers, masses, adducts, fragments, lipid ontology terms, and reactions. For each of these categories, LipidLibrarian aggregates the results from all databases into a consistent layout, such that it is traceable from which source each value originates. This layout also enables the user to quickly assess if the databases contain differing or conflicting information.

In summary, LipidLibrarian enables effortless, comprehensive, and automated searching for lipid information. By preventing tedious research, comparison, and aggregation of lipid information, it accelerates the research workflow, making it a meaningful tool for the scientific community.

Sputum Lipid Profiling in Tuberculosis for Investigating Correlation between Treatment Response and Metabolization of Tuberculostearic Acid Containing Phosphatidylinositols

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Tuberculosis (TB) is a major global health burden, with the WHO reporting about 1.6 million deaths and about 11 million infection cases in 2021. Especially due to the increasing occurrence of drug resistant *Mycobacterium tuberculosis (Mtb)* strains, the causative agent of TB, treatment becomes more difficult. On this basis, analysis of molecules that indicate infection status and therapy response are needed to individualize patients' treatment. Extensive efforts have been devoted for the understanding of host-pathogen interactions in TB and here we explore lipid profiling in sputum samples of TB patients. The complex cell wall of *Mtb* is equipped with high amounts of lipids having unique structural features that are not present in eukaryotic lipidomes. Here we investigate the potential of lipidomic analyses to gain further insight into the metabolization of tuberculostearic-acid (TSA, 19:0) containing phosphatidylinositols (PI) in sputum during antibiotic treatment[1]. For that we first analyzed the stability of PI 16:0_19:0 in cell culture models of *Mtb* infection to determine rates of incorporation and modification by the host cells. In a small well-described patient cohort (n = 10) that was treated with a standard regimen (isoniazid, rifampicin, ethambutol and pyrazinamide), we analyzed the lipid profiles at baseline, after 2 weeks and 2 months.

In our study, we integrate antibiotics measurements from sputum and blood plasma, targeted lipid quantitation of TSA – containing PIs in sputum and its cell fraction with overall lipidome profiling to gain insights in treatment response and its impact on the lipid metabolic interaction between host and pathogen. For that we applied the well-established shotgun lipidomics pipeline based on nanoESI-MS/MS (Q Exactive™ Plus (Thermo), Triversa Nanomate (Advion)). First results show, a good correlation between PI 16:0_19:0 (TSA) and the mycobacterial load in sputum during treatment as well as a shift towards higher portion of TSA-containing lipid metabolites after treatment. With this proof-of-principle study we further investigate the potential of lipid based treatment monitoring in TB.

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Using lipidomics to elucidate the effects of breast cancer chemotherapy on brain and blood lipids

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Objectives: Chemotherapy produces long-term cognitive impairment (CICI) in >30% of those receiving solid tumor treatment. Our group has worked to identify which brain lipids are adversely impacted following chemotherapy treatment in a murine model, and whether changes in the same lipids are observed in the blood of breast cancer patients prior to, during, and after chemotherapy treatment.

Methods: Our previous analyses using an untargeted UHPLC-QTof lipidomics approach found significantly increased omega-9 fatty acid concentrations in the brain cortex of ovariectomized mice following treatment with doxorubicin + cyclophosphamide, as compared to a vehicle control. A follow-up study measured omega-9 and structurally analogous fatty acids in human plasma following breast-cancer treatment, to determine if they might serve as biomarkers and/or nutritional targets during treatment. Serum samples were collected from patients (n=51) at ≥2 of 3 visits: immediately prior to standard adjuvant and neo-adjuvant chemotherapy for breast cancer (baseline), prior to the third cycle of chemotherapy, and 6 months after chemotherapy termination. Lipophilic extracts of serum were analyzed using ultra-high performance liquid chromatography-tandem mass spectrometry (UHPLC-MS/MS) to quantify eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), linoleic acid, oleic acid, mead acid, gondoic acid, erucic acid, nervonic acid and PE(P-18:0/22:6) (plasmalogen). Linear models assessed the impact of visit on fatty acid concentrations, with a fixed intercept used for each subject. A $P < 0.05$ for chemotherapy was considered significant. Similar models are being employed to determine if concentrations of these fatty acids are correlated with measures of memory and cognition.

Results: Chemotherapy significantly increased plasma concentrations of gondoic, erucic, nervonic acids and plasmalogen (with no effect on the other fatty acids tested). Six months following chemotherapy, concentrations of gondoic, erucic, nervonic acids and plasmalogen approached baseline concentrations.

Conclusion: Lipidomics is a useful approach to identify how breast cancer chemotherapy influences both brain and blood lipid concentrations. In this study, breast cancer chemotherapy increased omega-9 fatty acids, a class of lipids also increased in subjects with increasing severity of cognitive impairment and Alzheimer's disease. Future investigations will determine if these compounds may be driving CICI, and whether they may serve as nutritional targets for dietary interventions prior to or during chemotherapy treatment.

Untargeted hair lipidomics: Comprehensive evaluation of the hair-specific lipid signature and considerations for retrospective analysis

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Lipidomics investigates the composition and function of lipids, typically employing blood and tissue samples as the primary study matrices. Hair has recently emerged as a potential complementary sample type to identify biomarkers in early disease stages and retrospectively document an individual's metabolic status due to its long detection window. However, the limited coverage of lipid profiling presented in previous studies has hindered its exploitation.

This study aimed to evaluate the lipid coverage of hair using an untargeted liquid chromatography-high resolution mass spectrometry lipidomics platform. Two different three-step exhaustive extraction experiments were conducted using a hair metabolomics one-phase extraction technique and the two-phase Folch extraction method, considered the gold standard for lipid extraction in biological matrices, respectively. The hair lipidome was compared to blood, and sebum lipidome to understand its role in reflecting health and disease status.

The lipidomics workflow improved hair lipid coverage, extracting 99 species with the one-phase extraction method and 297 with the Folch method across six categories, yielding 91% of extractable lipids. Several lipids in hair were reported for the first time, including N-acylglycines, diradylglycerols, oxidized fatty acids, and coenzyme Q10. The study suggests that hair lipids are not solely derived from *de novo* synthesis in hair, but are also incorporated from sebum and blood, making hair a valuable matrix for clinical, forensic and dermatological research. The improved understanding of the lipid composition and analytical considerations for retrospective analysis offers valuable insights to contextualize untargeted hair lipidomic analysis and facilitate the use of hair in translational studies.

Identifying Double-Bond Positions of Phospholipids in Mouse Liver by Using Simultaneous Positive/Negative Ion Switching Analysis of LCMS-9050 and OAD-MS/MS

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Introduction

Lipids, a class of biomolecules, play a significant role in physiological systems. Non-targeted lipidomics by High-Resolution Accurate Mass Spectrometers (HRAM) is currently one of the major analytical workflows for clinical research such as biomarker discovery, as well as basic studies. However, conventional methods were unable to identify double bond positions in lipids without bias. A brand-new technique, Oxygen Attachment Dissociation (OAD), provides diagnostic fragment ions enabling the assignment of double bond positions. Identifying these positions by OAD-MS/MS is expected to contribute to our understanding of lipid metabolism. In this study, we focused on the fragmentation derived from phosphatidylcholine (PC) by using OAD-MS/MS and identified the chemical structures.

Methods

All experiments were performed using an ESI Q-TOF LCMS-9050 (Shimadzu, Kyoto, Japan). OH and O radicals generated by a compact microwave-driven radical source were introduced into the collision cell (Q2) through a quartz tube to obtain the OAD-MS/MS spectrum [1][2]. The gas pressures inside and outside the collision cell were maintained below 0.1 and 3×10^{-3} Pa, respectively, which were within the normal range for QTOF-MS. Shimadzu's Nexera LC system was used for the separation of lipids in the extracts of mouse liver.

Preliminary Data

Extracts from mouse livers were subjected to analysis with LCMS-9050, which allows simultaneous positive/negative ion switching analysis. All data were acquired by the data-dependent acquisition (DDA). The positive ion mode was used to generate OAD-MS/MS-based spectra to determine the positions of the double bonds, and spectra based on Collision Induced Dissociation (CID) were generated to confirm the lipid classes. The negative ion mode was used to generate spectra based on CID to confirm the length of fatty acid. The length of the fatty acid couldn't be estimated only in the positive ion mode, but simultaneous analysis with the negative ion mode enabled identification of the chain length. Several PCs in the mouse liver were assigned by MS-DIAL. The neutral loss fragment obtained from the negative ion mode helped to determine the length of the fatty acid, and OAD-MS/MS spectra obtained from the positive ion mode were used to identify double bond positions in PCs. From the combination of processing the data, PC 18:3_18:1 was assigned, and we successfully demonstrated that it has gamma-linolenic acid in the chemical structure instead of alpha-linolenic acid. Other PCs that have the same mass-to-charge ratio were also identified for chain length and double bond positions.

HERPES SIMPLEX VIRUS TYPE 1 REMODELS NEUTRAL LIPID METABOLISM AND INDUCES LIPID DROPLET ACCUMULATION IN DENDRITIC CELLS

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Herpes simplex virus type 1 (HSV-1) infects dendritic cells (DCs), antigen-presenting immune cells that are key for initiating and regulating host antiviral responses. HSV-1-infected DCs display hampered maturation, poor T cell activation capacity, reduced migration to draining lymph nodes, and ultimately, undergo apoptosis. Here, we report that HSV-1 significantly modulates the neutral lipid metabolism in infected DCs and induces lipid droplet (LD) accumulation in these cells.

HSV-1 exposure to dendritic cells was evaluated at 12 and 18 hours post infection (hpi). Extraction of lipids was achieved by Matyash method. LC-MS based lipidomics was performed in reverse phase chromatography (UHPLC) tandem quadrupole-time of flight (Q-TOF) mass spectrometry, the result analyses were processed with MSDIAL for signal alignment and with Metaboanalyst for multivariate processing and significant features detection. The features were checked with mass spectrometry databases such as HMDB and Lipidmaps, and in silico fragmentation performed for structural elucidation with SIRIUS and CFM-ID. Features annotation was reported by following the mass spectrometry initiative standard (MSI).

This lipidomic analysis identified 58 specific signals associated with types of lipids at 12- and 18-hpi in HSV-1-infected samples. An enrichment analysis of the different lipid classes detected in HSV-1-infected DCs revealed a preferent increase in the abundance of ceramides, cholesterol and derivates, DAGs, acylcarnitines, TAGs, sterol esters and sphingolipids at 12-hpi, while glycerolipids, TAGs, DAGs, fatty esters, sphingolipids, steroids, ceramides, glycerophosphoserines, glycerophosphoethanolamines, and sterols increased in abundance at 18-hpi. An interactome network evidenced three main nodes that were increased in HSV-1-infected DCs, 2 of them associated with neutral lipid metabolism (Cholesterol-Cholesterol ester node and Glycerolipids-TAG node), and a node related to palmitoyl-CoA-L-palmitoylcarnitine.

Optimization of a lipidomic method for Chilean olive oil using HPLC-DAD-QTOF-MS/MS.

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Olive oil (OO) is one of the foods consumed daily in the Mediterranean diet [1], and only its extra-virgin quality (EVOO) is beneficial for human health, as it reduces the prevalence of metabolic syndrome, diabetes mellitus, and cardiovascular diseases, among others [2]. This is because of its composition, which is divided into saponifiable fractions, which is mainly composed of tri, di and monoacylglycerols (TAGs, DAGs, and MAGs), free fatty acids (FFA), and phospholipids (PLs), representing almost 98% of its weight, and an unsaponifiable fraction that only represents 2% [3].

Lipids are closely related to organic disorders, and it is important to study their composition in OO. [4]. Therefore, based on the study by Cajka and Fiehn (2017) [5], a lipidomic analysis method using HPLC-QTOF for Chilean olive oils was developed to optimize the sample volume, injection volume, and gradient. The sample and injection volumes were optimized by performing a Central Composite face-centered design (CCF), using the number of total features as responses. An optimal sample volume of 0,8 µL and injection volume of 6 µL were obtained with a total number of features of 1890.

Prior to analysis, sample preparation is necessary, where a biphasic liquid-liquid extraction with MTBE/MeOH/H2O (10:3:2.5, v/v/v) is performed. The organic phase is extracted and directly analyzed by HPLC-DAD-QTOF-MS/MS using a Phenomenex Kinetex C18 100 x 4.6 mm 2.6 µm column, with a mobile phase gradient of acetonitrile/water (60:40 v/v) and isopropanol/acetonitrile (90:10 v/v) at a flow rate of 0.6 mL/min.

Using this method, it has been possible to identify certain lipids present in Chilean olive oil, mainly triglycerides and diglycerides.

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BLOOD CLOT AND SERUM LIPIDOMICS IN ISCHEMIC STROKE

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Introduction: Lipids are highly involved in blood clot formation as well as in cerebral post-ischemic inflammation and stroke pathologies in the brain. Stroke is one of the leading causes of death and disability globally with 80% of all strokes being ischemic strokes. The aim of this study was to perform lipidomic profiling of blood clots that caused ishemic stroke and to compare it to the patients' serums and healthy controls serums.

Experimentals: A targeted lipidomics approach was performed on blood clots removed from the brain by mechanical thrombectomy, the serums of the same patient and healthy controls' serums using the Absolute IDQ p400 kit (Biocrates Life Science AG). Samples were applied on the 96-well plate system for protein removal, internal standard normalization and derivatization. Metabolite extracts were analysed by combined LC-MS/MS and FIA-MS/MS analysis on a Thermo Orbitrap Q Exactive Plus UHPLC-MS/MS. Identification and quantification of metabolites were made using the Biocrates MetIDQ software (Biocrates Life Science AG, Innsbruck, Austria).

Results: Targeted lipidomics analysis resulted in a total of 108 lipid species identified in blood clots (BC) samples, 164 lipids in the corresponding patients' serums (PS) and 163 lipids in the serum samples of healthy control subjects (HS). Different classes of lipids such as ceramides, sphingomyelins, glycerides, glycerophos-pholipids, cholesteryl esters and acylcarnitines were identified in all three groups of samples. Ceramides (42:2), (40:1), (38:1) and (34:1) were present in much higher concentrations in BC samples than in the PSs. Sphingomyelin SM(34:1) was detected in the highest concentration in all samples, followed by SM(36:1), (40:1) and (42:2), with (40:1) being present in BC samples in two times higher concentration than in the serum samples. Lysophosphatidylcholines LPC(18:1) and (18:0) were present in BC samples in five times lower concentrations than in the serum samples, while LPC(18:2) was present in two times lower conc. in patient serum than in healthy serums. Phosphatidylcholines PC(34:2) and (36:4) were present in the highest concentrations in HS samples, followed by lower conc. in PS and up to 5 times lower conc. in BC samples. Triglycerides TG (52:3) followed by (52:2) and (52:4) were present in the highest conc. in HS, aprox. two times lower in PS and up to 20 times lower in BC samples compared to HS samples. Cholesteryl esters CE(18:2) were present in the highest conc. in all samples, having 20 times higher conc. in HS and PS samples than in BC samples.

Conclusion: Lipidomic profiling of blood clots in comparison to serum lipid composition may elucidate the roles of different lipid classes in coagulation, which could help in better understanding the pathogenesis of ischemic stroke and suggest new potential candidates for early diagnostic and therapeutic biomarkers of ischemic stroke.

An LC-HRIM-MS pipeline for elucidating the isomeric lipidome

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Analyzing complex lipid samples is complicated by the presence of numerous isomers, which are challenging to fully characterize using traditional liquid chromatography-mass spectrometry (LC-MS) workflows. The introduction of high-resolution ion mobility (HRIM) techniques, such as structures for lossless manipulations (SLIM), enables rapid, gas-phase separation of lipids with resolving powers over 250, facilitating the identification of biologically relevant lipid isomers uncharacterized in prior analyses. In this work, we developed a full LC-HRIM-MS workflow for lipidomic analysis enabling deeper characterization of samples via multidimensional separations. Source parameters, LC gradient, HRIM trap/release and separation parameters, and DDA fragmentation settings, among others, were tuned for optimal lipid response and separation. Lipid standards were chosen to cover multiple lipid classes of interest in the omics community that are expected to be present in serum, including PC, PE, TAG, SM, and Cer species. Commercial standards, including a full suite of isomerically pure standards containing six *sn* and double bond position isomers of PC 16:0/18:1, were analyzed to determine their CCS values and construct CCS databases in both Lipostar and Skyline software. The CCS database in Lipostar is intended for untargeted analysis, utilizing CCS in its identification database matching and scoring algorithms. When combined with the resolving power of HRIM, closely related lipid isomeric species with CCS differences down to ~0.4% can be separated and therefore identified to a higher level of structural specificity than using LC-MS/MS alone. Skyline, on the other hand, is primarily a targeted analysis platform used for quantitative analysis. We demonstrate the power of the application using NIST SRM 1950, by increasing the number of identifications of isomeric species not revealed by standard LC-MS approaches resulting in an overall improvement in lipid separation, identification, and quantitation.

Metabolomics profiling of signaling lipids: a comprehensive UHPLC-MS/MS method for quantitative analysis of oxidative stress and inflammatory markers applied to NIST SRM 1950

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Signaling lipids (SLs) are bioactive lipids that mediate a variety of signaling cascades such as cell proliferation and differentiation, innate immunity and inflammation as well as in normal physiological functioning (e.g. pregnancy, exercise and appetite). Emerging evidence showing the pathophysiological relevance of SLs necessitates their metabolic profiling and biological elucidation while the dramatic differences in endogenous abundances across lipid classes as well as multiple isomers within the same lipid class makes the development of a generic analytical method challenging.

In this study, we developed a fast and comprehensive targeted ultra-high performance liquid chromatography–tandem mass spectrometry (UHPLC-MS/MS) method for profiling SLs. The platform enables analyses of 261 metabolites covering oxylipins (isoprostanes, prostaglandins and other oxidized lipids), free fatty acids, lysophospholipids, sphingoid bases (C16, C18), platelet activating factors (C16, C18), endocannabinoids and bile acids. The metabolites covered in this method can provide readouts for the metabolic signaling mediators underlying (patho)physiological conditions pertaining to inflammation, oxidative stress and immune-dysfunction, whereas the bile acids provide insights into the gut microbiome as well as hormonal and metabolic control. Various validation parameters including linearity, limit of detection, limit of quantification, extraction recovery, matrix effect, intra-day and inter-day precision were used to characterize this method. The NIST Standard Reference Material for human plasma (NIST SRM 1950) was used to perform metabolite quantitation and the results were compared with reported or certified values. We report concentrations of 37 SLs in NIST SRM 1950 which were not previously reported and are informative for lipidomics researchers.

Ultraviolet photodissociation (UVPD) mass spectrometry for structural characterization of lipids in biological matrices on chromatographic time scales

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Introduction

Complete structural characterization of lipids is an essential component of lipidomics but remains challenging as, commonly used activation methods, such as HCD and CID, do not generate fragments to discern regioisomers and pinpoint the double bond positions within the acyl chains. Ultraviolet photodissociation (UVPD) energizes ions via absorption of high-energy photons generated from an UV laser source. This increases the internal energy of a selected precursor ion (electronic excitation), until there is sufficient internal energy present to overcome the barrier for dissociation, therefore generating fragments. Here we show the use liquid chromatography-based separation of lipids with the use of hybrid dissociation strategies using conventional and UVPD MSn fragmentation for structural characterization of specific classes of lipids in biological matrices.

Methods

Lipid standards and bovine liver lipid extracts were purchased from Avanti Lipids. NIST SRM 1950 plasma sample was purchased from Sigma. Lipids were extracted from a variety of biological matrices including plasma and plants using Folch method. Lipids were separated on a Thermo Scientific™ Accucore™ C30 column connected to a Thermo Scientific™ Vanquish™ Horizon system. Data were acquired on a Thermo Scientific™ Orbitrap IQ-X Tribrid Mass Spectrometer equipped with UVPD (213 nm laser). The use of a tribrid allows the use of hybrid strategy utilizing higher-energy C-trap dissociation (HCD) and collisionally activated dissociation (CAD) with UVPD for fragmentation of lipids up to MS^n level. Thermo Scientific™ Mass Frontier™ and LipidSearch™ 5.1 software were used for lipid annotation and data processing.

Preliminary Data

UVPD allows access to new dissociation pathways complementary to more conventional ion-activation methods and has been shown to generate fragments useful for complete structural elucidation of lipids. In this study, initially lipid standards were infused into the mass spectrometer using hybrid strategy for fragmentation utilizing HCD/CID for MS2 followed by UVPD based MS3 fragmentation of specific product ions generated by the neutral loss of head group. The optimal activation time required for UVPD to generate diagnostic fragments for regioisomers and double bond positions of acyl chains was determined for lipid class phosphatidylcholines (PC) and triacylglyceride (TAG). 150 ms-300 ms activation time was found to be sufficient for characterization of regioisomers.

The lipid standards and extracts were separated using LC. The data was acquired using mass spectrometer with optimized parameters for fragmentation. Thermo Scientific AcquireX™ strategy, with iterative precursor workflow, was used for analyzing the samples. This workflow automatically generates an exclusion list for background compounds to focus on the fragmentation of extracted lipids. Iterative injections of the sample update the exclusion list and hence even low intensity lipid ions can be fragmented. The use of AcquireXTM allows efficient use of the mass spectrometer duty cycle for fragmentation of ions in LC time scale.

Lipids present in the biological matrices were annotated for lipid class and the acyl chain composition using LipidSearch™ 5.0 software. Manual annotation as well as using Mass Frontier™ was done on the UVPD fragmentation data of specific PC and TAG lipids to determine the regioisomers as well as the double bond positions on the acyl chains. Using the hybrid dissociation strategy, we can do complete structural characterizations of lipids in biological matrices on LC time scale.

Novel Aspect

Structurally defined molecular lipid annotations in biological matrices using LC-MS with a hybrid dissociation strategy.

Nano-LC-MS based lipidomics for Single Cell Applications

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Introduction

Lipidomics is the comprehensive analysis of molecular lipid species. Separation of lipids using liquid chromatography followed by analysis by mass spectrometry has become the method of choice for lipidomic studies. Many biological specimens including single cell extracts are limited in sample amounts and hence require highly sensitive analysis methods. Conventional LC-MS based lipidomics does not offer the sensitivity required for the comprehensive lipidomic analysis of these samples. Nano-LC-MS offers high sensitivity but is technically challenging to implement in terms of robustness and reproducibility. This work describes the development and optimization of nano-LC-MS using micropillar array-based columns for robust and reproducible lipidomic analysis.

Methods

Lipid standards and bovine liver lipid extracts were purchased from Avanti Lipids. For conventional LC-MS, lipids were separated on a Thermo Scientific™ Accucore™ C18 column connected to a Thermo Scientific Vanquish™ Horizon HPLC system. For nanoLC-MS lipids were separated on a Thermo Scientific µPAC™ - column connected to a Thermo Scientific VanquishNeo UHPLC system. Data were acquired on a Thermo Scientific Orbitrap IQ-X Tribrid Mass Spectrometer equipped with UVPD (213 nm laser). Thermo Scientific LipidSearch™ 5.0 software was used for lipid annotation and data processing.

Preliminary Data

Dilution series of lipid standards and bovine liver lipid extracts were analyzed using both the conventional LC-MS as well as nano-LC-MS. µPAC columns are built by precise micromachining chromatographic separation beds into silicon. This leads to lower back pressure, high resolution, robustness and high reproducibility. The flow rates and run time for the nLC were optimized for greater separation and resolution of the eluted lipid species. Repeated injections of the same sample- showed a maximum retention time drift of 0.1 minutes.

We further benchmarked the developed nLC system to the conventional high flow HPLC/ESI MS system in terms of lipidome coverage and sensitivity. We also found an increase in sensitivity of all lipid classes going up to almost 10 times for certain lipid species such as acyl-carnitines. There was also an increase in dynamic range of almost 2 orders of magnitude. For similar amounts of injected lipids, we were able to annotate almost twice the lipid species in nLC compared to conventional LC separation using LipidSearch software.

Novel Aspect

nLC-MS based method for lipidomics is developed using micropillar array-based columns to approach sensitivities required for single cell lipidomic applications.

Strategies for the reliable analytical discrimination between plasmanyl and plasmenyl lipid species in lipidomics experiments

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In order to enable life as we know it, a wide variety of different lipids are required. Among these, ether lipids represent an important class, whose characteristic ether bond can be present as plasmanyl or plasmenyl (=plasmalogens) form. The differentiation of plasmalogens from other ether lipids is both, of great significance due to their distinct physicochemical properties and a great analytical challenge in lipidomics experiments. In our work, we explore the analytical possibilities to discriminate between plasmanyl and plasmenyl lipids.

Tissue samples of wild-type and knockout mice deficient in plasmalogen biosynthesis were utilized as model systems. Samples were analyzed by means of reversed phase liquid chromatography-tandem mass spectrometry (LC-MS) with carefully optimized chromatographic conditions. This allowed us to systematically explore retention time differences, which can be used as a main discriminatory parameter to distinguish between the isomeric plasmanyl/plasmenyl lipid pairs. This approach can enable accurate LC-MS based lipid identification and quantification.

As an orthogonal method, we next investigated the behavior of ether lipids in ion mobility spectrometry (IMS) coupled with LC-MS. IMS separates ions based on their mobility in a gas phase. The respective collision cross section (CCS) thereby defines an ions size in respect to and electrical field and an opposed inert gas stream, which in analogy to the retention time differed between isomeric plasmanyl/plasmenyl lipid pairs.

A combination of both approaches will in the near future enable a rapid, straight forward and exact identification of plasmanyl and plasmenyl lipids in untargeted lipidomics experiments. This in turn will advance ether lipid research by generating deep insights into the respective roles of plasmanyl and plasmenyl lipids in cellular metabolism and disease mechanisms in the future.

Matrix effects of plasma in untargeted LC-MS

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Lipids play a crucial role in all biological processes. Recognizing the biological significance of lipids, lipidomics has emerged as major field in analytical chemistry.

The combination of liquid chromatography (LC) with electrospray ionization (ESI) and high-resolution mass spectrometry (HRMS) is one of the most powerful tools for simultaneous monitoring of a diverse range of lipids extracted from biological samples. However, the matrix of these samples can introduce interference such as ion suppression during ESI.

In this study, we explore the impact of matrix effects on untargeted LC-HRMS/MS (Q Exactive HF) analysis. We assess the efficiency of lipid extraction from plasma using a set of isotopically labeled internal standards (IS, SPLASH mixture). Additionally, we conduct a comprehensive investigation of ion suppression by postcolumn infusion of the internal standards.

The lipids were extracted from plasma using liquid-liquid extraction with methyl *tert*-butyl ether and methanol. Chromatographic separation was carried out on a C18 reversed-phase column (100 x 2.1 mm, 1.7 μm). Following ionization by ESI in both negative and positive mode, mass spectrometric data were acquired in Full MS/data-dependent MS^2 mode.

Spiking experiments demonstrated a good extraction efficiency and acceptable ion suppression (overall recovery of isotopically labeled IS > 85%). It was observed that apparent losses were attributed to ion suppression caused by coeluting lipids. Furthermore, the analysis revealed that the signal for two individual IS was diminished to zero over several minutes during the analysis. Our findings indicate that this phenomenon is not due to ion suppression but a result of interference from matrix compounds with similar *m/z* values. We conclude, that the Fourier transformation performed by the orbitrap assigned the signals of these IS and coeluting matrix to a single *m/z* value causing the deletion of their signals.

Accurate ceramide quantification without fragmentation bias by non-linear models

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Quantitative sphingolipid analysis is crucial for comprehending the roles of these bioactive molecules in various physiological and pathological contexts [1]. Molecular sphingolipid species are typically quantified using sphingoid base-derived fragments relative to a class-specific internal standard. However, the commonly employed 'one standard per class' strategy fails to account for fragmentation differences presented by the structural diversity of sphingolipids. To address this limitation, we have developed a novel approach for quantitative sphingolipid analysis. Starting from a previously developed LC-ESI-MS/MS-based approach for the comprehensive analysis of sphingolipids in biological samples, we focused on optimizing the quantification in terms of post-acquisition data correction. To avoid overlaps of species overall differing by only one double bond, in addition to chromatographic separation, a fragment of the LCB was used for quantifying. Using only one internal standard for the entire lipid class, we developed a fragmentation model for ceramides. This approach uses the information on double bonds and hydroxy groups of the long-chain base as well as fatty acid, respectively, for a rough calculation of the correction factor and the chain length for a more precise one. The determined response factors are based on experimental data and are independent of the employed instrumentation, collision energies or matrix, and may be extended to another internal standard or different sphingolipid classes. To automatize data processing after acquisition, such as calculating species-specific correction factors, a workflow was developed using the software tool "Konstanz Information Miner" (KNIME) [2]. Overall, the workflow has proven suitable for processing data from ceramide analyses of complex biological samples, such as fat cells [3], giving the same quantitation results, as manually obtained from the same data set, in 1/100 of the time.

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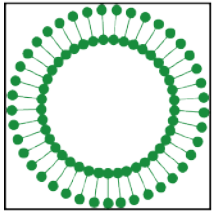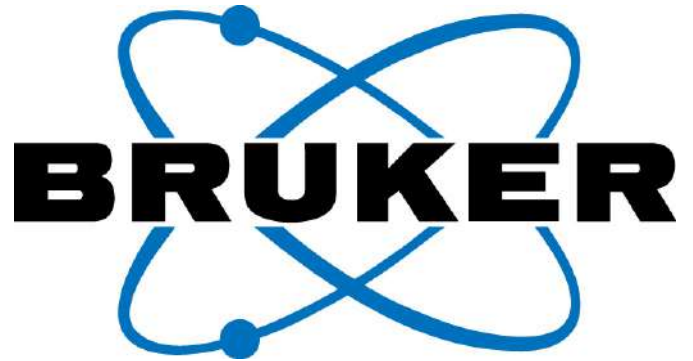
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