LIPIDOMICS FORUM

10th Lipidomics Forum
September 14th - 17th 2025

AbstractBook

Research Center Borstel
Leibniz-Center for Medicine and Biosciences

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LDA2 Workshop

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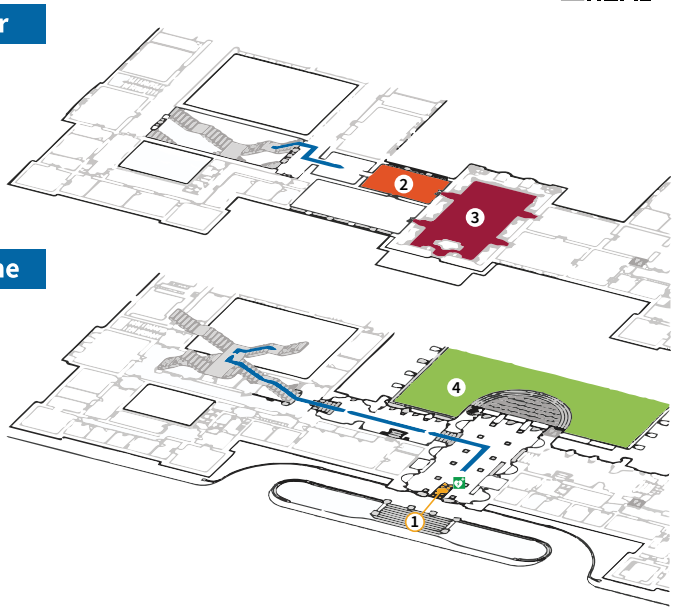
Orientation Plan

University Main Building
Universitätsring 1, 1010 Vienna

1st Floor

Mezzanine



Heurigen Evening
sponsored by the ILS & EuroFed
free for every conference participant
Feuerwehr Wagner
Grinzinger Straße 53, 1190 Vienna

Conference Dinner
for registered dinner participants only
Fuhrgassl-Huber
Neustift am Walde 68, 1190 Vienna

1 Entrance
Universitätsring 1, 1010 Vienna

2 Registration & Cloakroom
via Staircase 1 (Stiege 1)

3 Main Ceremonial Hall
for the Opening Keynote

4 Arcaded Courtyard
for the Get Together

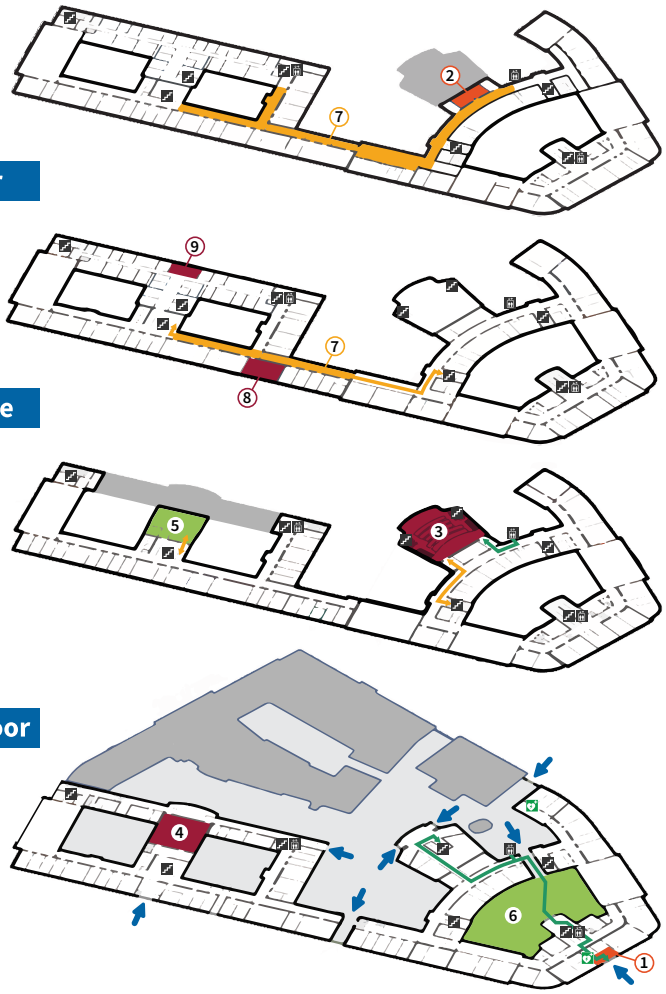
Faculty of Chemistry
Währinger Straße 38, 1090 Vienna

2nd Floor

1st Floor

Mezzanine

Ground Floor



1 Main Entrance & Registration
Währinger Straße 38, 1090 Vienna

2 Cloakroom
Seminar Room 1 (SR1)

3 Lecture Hall 1
Carl Auer von Welsbach Lecture Hall (HS1)
all Keynotes, Tutorials, and Talks

4 Lecture Hall 2
Joseph Loschmidt Lecture Hall (HS2)

5 Vendor Exhibition Hall & Break Room
Student Center Hall (Studierendenzentrum),
for coffee, lunch, snacks, and beverages

6 Courtyard
for coffee & lunch breaks

7 Poster Exhibition Hallways
see the poster plan for more details

8 LIFS Workshop
Seminar Room 2 (SR2)

9 LDA2 Workshop
Dean's Office Conference Room, 2153-W

Schedule

Sunday | **September 14**

9:00 – 15:00 **WORKSHOPS | FACULTY OF CHEMISTRY**
LIFS (SR2) | LDA2 (Room 2153-W)

15:00 – 16:00 **REGISTRATION OPENING | UNIVERSITY MAIN BUILDING**

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

16:15 – 17:00 **OPENING KEYNOTE** | Chair: Robert Ahrends
Sandvig, Kirsten
T01 **Cellular Membrane Dynamics and Lipid Composition**
University of Oslo, Norway 11

17:15 – 21:00 **GET TOGETHER**
Arcaded courtyard, Drinks and Snacks

Monday | **September 15** | Faculty of Chemistry

8:00 – 9:00 **REGISTRATION**

9:00 – 9:45 **TUTORIAL** | Chair: Dominik Schwudke (9:00 – 12:45)
Skotland, Tore
T02 **Biological membranes and lipid analyses**
Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital and Centre for Cancer Cell Reprogramming, University of Oslo, Norway 12

9:45 – 10:30 **KEYNOTE**
Simcox, Judith
T03 **Harnessing diverse human populations to explore the function and regulation of plasma lipids**
University of Wisconsin-Madison, United States of America 13

10:30 – 11:00 **COFFEE BREAK**
Served in the Student Center Hall (Studierendenzentrum) and in the courtyard

11:00 – 11:45 **KEYNOTE**
Ejsing, Christer S.
T04 **Profiling the metabolic fates of dairy lipids in health and disease**
University of Southern Denmark, VILLUM Center for Bioanalytical Sciences, Odense, Denmark. 14

11:45 – 12:45 **SESSION 1 (METABOLISM 1)**
11:45 PRIZE Choong, Laura L.Y.
T05 **Investigating the effects of a novel ceramide synthase inhibitor on the localisation of subcellular ceramides**
Cellular Bioenergetics Lab, Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia 15

12:05 Brunner, Sarah

T06 **Mitochondrial phosphatidylethanolamine (PE) synthesis essentially regulates whole body lipid metabolism and metabolic health**

University Hospital Regensburg, Germany 16

12:25 Jové, Mariona

T07 **Plasma hexocylceramide and ether lipid enrichment as hallmark of human extreme longevity**

University of Lleida-Biomedical Research Institute of Lleida, Spain 17

12:45 – 13:45 **LUNCH | Sciex Lunch Seminar** | Chair: SCIEX Associates and Users

Lecture hall 2

13:45 – 14:30 **KEYNOTE** | Chair: Evelyn Rampler (13:45 – 16:45)

Ecker, Josef

T08 **Beyond the gut: How the microbiome regulates whole-body lipid metabolism**

Functional Lipidomics and Metabolism Research, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany 18

14:30 – 15:30 **SESSION 2 (METABOLISM 2)**

14:30 Merciai, Fabrizio

T09 **Beyond alpha-fetoprotein: Development of a targeted lipidomics assay for accurate discrimination of HCC from other liver diseases**

University of Salerno, Italy 19

14:50 Mota-Martorell, Natalia

T10 **The circulating bioenergetic lipotype as a distinctive signature in Centenarians**

Metabolic Pathophysiology Research Group, Department of Experimental Medicine, University of Lleida-IRB Lleida, Lleida, Spain. 20

15:10 Munjoma, Nyasha

T11 **Shotgun lipidomic workflow for single cell analysis using single and multipass cyclic ion mobility**

Waters, United Kingdom 21

15:30 – 16:00 **COFFEE BREAK**

Served in the Student Center Hall (Studierendenzentrum) and in the courtyard

16:00 – 16:45 **KEYNOTE**

Koeberle, Andreas

T12 **Revealing cancer vulnerabilities in stress adaptation and ferroptosis through functional lipidomics**

University of Graz, Austria 22

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

Poster Exhibition Hallways

19:30 **HEURIGEN EVENING by ILS & EuroFed**

Weingut Feuerwehr Wagner - Grinzinger Straße 53, 1190 Vienna
Free for all conference participants

Tuesday | **September 16** | Faculty of Chemistry

9:00 – 9:45 **KEYNOTE** | Chair: Robert Ahrends (9:00 – 14:25)

Abu-Remaileh, Monther

T13 **Neurodegeneration: Intra-Lysosomal Lipid Metabolism in the Driver's Seat**

University Stanford, United States of America 23

9:45 – 10:30 **KEYNOTE**

Hatcher, Nathan

T14 **Lipid Metabolic Defects Underlie Parkinson's Disease Pathology**

Merck & Co., Inc., Rahway, NJ, USA 24

10:30 – 11:00 **COFFEE BREAK**

Served in the Student Center Hall (Studierendenzentrum) and in the courtyard

11:00 – 12:40 **SESSION 3 (NEUROLIPIDOMICS)**

11:00 PRIZE Nguyen-Tran, Thao

T15 **Pathway strength score computation and utilization as a lipid metabolic index to assess novel transglycosidase function in GBA1-PD patients**

Neurolipidomics Lab, India Taylor Lipidomic Research Platform, and Department of Chemistry and Biomolecular Sciences 25

11:20 Obis, Elia

T16 **Region-Specific Lipid Remodeling in Alzheimer's Disease: A Spotlight on White Matte**

Biomedical Research Institute of Lleida (IRBLLeida), Spain 26

11:40 Bennett, Steffany

T17 **The metabolic cost of the lipidomic network-level response to ASAH1 mutation differentiates and provides new therapeutic options for Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy from Farber's Disease**

Neurolipidomics Laboratory and India Taylor Lipidomics Research Platform, University of Ottawa Brain and Mind Research Institute, Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, Department of Chemistry and Biomolecular Sciences, Centre for Catalysis Research and Innovation, University of Ottawa, Canada 27

12:00 Kollipara, Laxmikanth

T18 **LC-MS based analysis of phosphoinositides (PIPs) in metabolic, cardiovascular and degenerative disease**

Leibniz-Institut für Analytische Wissenschaften – ISAS – e.V., Germany 28

12:20 Ravi Deshpande, Rahul

T19 **A Highly Sensitive and Selective Targeted Method for Single Cell Lipidomics**

Thermo Fisher Scientific, San Jose, CA, USA 29

12:40 – 13:40 **LUNCH**

Served in the courtyard

13:40 – 14:25 **KEYNOTE**

Ekroos, Kim

T20 **Disentangling Complex Brain Lipid Phenotypes in Parkinson's Disease**

Lipidomics Consulting Ltd., Esbo, Finland 30

14:25 – 15:25 **SESSION 4 (TECHNOLOGY)** | Chair: Nina Troppmair (14:25 – 15:25)

14:25 PRIZE Kontiza, Anastasia

T21 **Lipidomics at the Single-Cell Level: Workflow Optimisation for Live Cell Analysis**

School of Chemistry and Chemical Engineering, Faculty of Engineering and Physical Sciences, University of Surrey – Guildford, GU2 7XH, U.K. 31

14:45 PRIZE Telle, Alice

T22 **Identification of double bond location in unsaturated free fatty acids of human sebum**

San Gallicano Dermatological Institute - IRCCS, Italy 32

15:05 Liebisch, Gerhard

T23 **Investigating the analytical response of triglycerides by flow-injection-analysis high-resolution mass spectrometry**

University Hospital Regensburg, Germany 33

15:25 – 15:55 **COFFEE BREAK**
Served in the Student Center Hall (Studierendenzentrum) and in the courtyard

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

19:00 **CONFERENCE DINNER**
Fuhrgassl-Huber - Neustift am Walde 68, 1020 Vienna

Wednesday | **September 17** | Faculty of Chemistry

9:00 – 9:45 **KEYNOTE** | Chair: Nicolas Gisch (9:00 – 12:10)
Schebb, Nils Helge
T24 **Technical recommendation for the quantitative analysis of oxylipins and new ideas for targeting them in oxidized phospholipids**
University of Wuppertal, School of Mathematics and Natural Sciences, Chair of Food Chemistry, Gaussstrasse 20, 42119 Wuppertal, Germany 34

9:45 – 10:30 **KEYNOTE**
Wheelock, Craig E.
T25 **Targeted multimodal analyses to spatially map octadecanoid metabolism in the lung**
Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden 35

10:30 – 10:40 **INTERNATIONAL LIPIDOMICS SOCIETY**
Direction and vision

10:40 – 11:20 **SESSION 5.1 (TECHONOLOGY / OXYLIPINS / BIOINFORMATICS)**
10:40 PRIZE Ding, Cong
T26 **Design-Optimized Extraction Unlocks Comprehensive Lipidome of Fucus vesiculosus**
University of Turku, Finland 36
11:00 PRIZE Wende, Luca Marcel
T27 **Quantification of Lipids in Human Plasma using MS-DIAL: A Comparison of HILIC and RP Chromatography in Untargeted LC-HRMS Lipidomics**
University of Wuppertal, Germany, Food Chemistry, Faculty of Mathematics and Natural Sciences 37

11:20 – 11:50 **COFFEE BREAK**
Served in the Student Center Hall (Studierendenzentrum) and in the courtyard
Please vote for the best Talk and Poster prize!

11:50 – 13:10 **SESSION 5.2 (TECHONOLOGY / OXYLIPINS / BIOINFORMATICS)** | Chair: Dominik Kopczynski (12:10 – 13:55)
11:50 Gerner, Christopher
T28 **Correlating abundance levels of lipid mediators with tissue proteomics data suggests pro-resolving functions of polyunsaturated fatty acids**
Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, Waehringer Str. 38, 1090 Vienna, Austria 38
12:10 Lamp, Leonida M.
T29 **LC=CL: A software tool for unmasking C=C positions in complex lipids by routine LC-MS/MS lipidomics**
Institute of Pharmaceutical Sciences, University of Graz, Graz, Austria 39

12:30 Tejera, Noemi

T30 **MetaboLights - Open Access Metabolomics and Lipidomics Data Repository**

EMBL/EBI, United Kingdom 40

12:50 Hunter, Jonathan E.

T31 **Metabolomics Hub: International Data Exchange and Data Representation Standards for Metabolomics & Lipidomics**

European Bioinformatics Institute (EMBL-EBI), European Molecular Biology Laboratory 41

13:10 – 13:55 **KEYNOTE**

Shevchenko, Andrej

T32 **New frontiers in shotgun lipidomics**

MPI of Molecular Cell Biology and Genetics, Dresden, Germany 42

13:55 – 14:25 **CLOSING SESSION** | Chair: Dominik Schwudke / Robert Ahrends

Prizes for best Talk and Poster awarded by Lipotype

14:25 **PICKUP LUNCHES**

Served in the courtyard

14:25 – 15:45 **DGMS INTEREST GROUP MEETING**

Lipid Analysis and Lipidomics

Abstracts of Talks

Cellular Membrane Dynamics and Lipid Composition

Sandvig, Kirsten[1]

[1] University of Oslo, Norway

T01

Vesicular uptake from the cell surface (endocytosis), intracellular transport, as well as release of extracellular vesicles, are not only dependent on membrane-associated proteins and protein coats, but can be modulated by the cellular lipid composition. To understand the role of lipids in membrane function we have during the years asked questions such as: What happens to a given cellular process when the lipid content of the membrane is changed? Can one actually predict what happens to lipid composition when enzymes involved in lipid synthesis are inhibited or precursors are added? The answer to the last question is: not always. One needs lipidomics, too little is known about cellular behavior and compensatory mechanisms to correctly predict the outcome. Surprises were obtained, an ether lipid precursor affected several lipid classes, inhibited transport from the Golgi apparatus to the ER, and increased secretion of extracellular vesicles. Also, the lipid composition in different cell types, membrane areas and organelles are not identical. Importantly, a parameter such as cell density may affect lipid composition. Thus, scientists with different background and competence are important to avoid mistakes and make the field move forward.

We have during many years been studying the different types of endocytosis both in nonpolarized and polarized cells, and the complexity has certainly been increasing when it comes to the number of processes known and molecules involved. However, conclusions about uptake mechanisms are not always based on sufficient knowledge. For instance, cholesterol is not only important for the structure of caveolae, small invaginations in the plasma membrane, so reducing the membrane content of cholesterol can inhibit other types of uptake such as macropinocytosis (formation of larger vesicles) and clathrin-dependent uptake. Also, care should be taken when lowering the cholesterol content with a compound such as cyclodextrin, as membranes may become leaky. Moreover, increasing the cholesterol content can have consequences such as inhibition of intracellular transport. In some of our studies we used probes such as Shiga toxin, which binds to the neutral glycolipid Gb3, and where we discovered that the uptake and retrograde transport to the Golgi apparatus, the ER and the nuclear membrane is dependent on lipid composition.

Biological membranes and lipid analyses

Skotland, Tore[1]

[1] Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital and Centre for Cancer Cell Reprogramming, University of Oslo, Norway

T02

I will start by shortly summarizing the main lipid classes found in biological membranes and the asymmetry of these lipids in such lipid bilayers. Small extracellular vesicles are useful for studies of the lipid composition of a biological membrane as they contain only one bilayer and thus are much simpler to study than cells which contain a large variation of intracellular organelles/membranes. I will describe how lipidomic studies of extracellular vesicles and molecular dynamic simulation studies based on these quantitative lipid analyses made us focus upon interactions between the two leaflets. Specifically, we focused on interactions between sphingolipids with very long chain N-amidated fatty acids in one leaflet and a specific phosphatidylserine species (PS 18:0/18:1) in the other leaflet, and the importance of cholesterol for these interactions. Such interactions could be important for the rapid transmembrane signaling observed when Shiga toxin binds to its receptor, the glycosphingolipid Gb3. Our work with lipid analyses of extracellular vesicles introduced us to a field where we observed that even publications in very good journal reported lipid species that most likely do not exist in the samples. These observations made us publish, together with scientists from the International Lipidomics Society, a commentary in Nature Reviews Molecular Cell Biology entitled “Pitfalls in lipid mass spectrometry of mammalian samples - a brief guide for biologists”, which will be discussed.

Harnessing diverse human populations to explore the function and regulation of plasma lipids

Simcox, Judith[1]

[1] University of Wisconsin-Madison, United States of America

T03

Plasma lipids are established fuel sources and indicators of metabolic health, but their role as signals of inter-organ communication is just beginning to be appreciated. A major barrier in understanding lipid function is transport, because lipids are hydrophobic, they localized to lipoprotein complexes or extracellular vesicles in the blood. Despite the critical role of lipoproteins in lipid routing, little is known about how lipoprotein association alters lipid function. Using the selective pressure of cold exposure in mice, which rapidly shifts plasma lipid composition, we determined that very long chain ceramides are increased with cold exposure and localized to high density lipoprotein (HDL) particles. These plasma ceramides are synthesized in the liver and taken up by brown adipocytes. Inhibition of ceramide synthesis by myriocin treatment, ablates ceramide production and causes cold intolerance. HDL-associated ceramides are transferred from HDL particles to brown adipocytes through the ApoA1 receptor, SR-B1, where ceramides regulate B3-adrenergic receptor phosphorylation to then increase energy expenditure. These results demonstrate an inter-organ cellular lipid signaling pathway that is dependent on lipoprotein localization to regulate energy expenditure. Understanding lipoprotein localization and the impact on function will allow us to probe the causal relationship between ceramides and metabolic disease.

Profiling the metabolic fates of dairy lipids in health and disease

Ejsing, Christer S.[1,2]

[1] University of Southern Denmark, VILLUM Center for Bioanalytical Sciences, Odense, Denmark.

[2] Cell Biology and Biophysics Unit, European Molecular Biology Laboratory, Heidelberg, Germany.

T04

The regulation of whole-body lipid metabolism plays a crucial role in maintaining cardiometabolic health, yet our understanding of how dairy lipids influence this remains limited. Using state-of-the-art lipidomic technologies, we investigate how milk lipids modulate metabolic and physiological processes. We further apply lipidomic flux technology to trace their metabolic fates, revealing roles in lipid transport and turnover. Our recent findings highlight the potential of a milk sphingolipid-rich MFGM/EV ingredient to ameliorate age-related metabolic dysfunction, and demonstrate how advanced lipidomics can support the evaluation of dietary strategies for maintaining metabolic health and mitigating disease risk.

Investigating the effects of a novel ceramide synthase inhibitor on the localisation of subcellular ceramides

Choong, Laura L.Y.[1,2], Hancock, Sarah E.[1,3], Nguyen, Amy[1], Dragutinovic, Iliya[4], Taylor, Elysha N.[4], Morris, Jonathan C.[4], Turner, Nigel[1,3]

[1] Cellular Bioenergetics Lab, Victor Chang Cardiac Research Institute, Darlinghurst, New South Wales, Australia
[2] School of Clinical Medicine, University of New South Wales, Sydney, New South Wales, Australia
[3] School of Biomedical Sciences, University of New South Wales, Sydney, New South Wales, Australia
[4] School of Chemistry, University of New South Wales, Sydney, New South Wales, Australia

T05 PRIZE

Ceramide, a sphingolipid, has a causal role in cardiometabolic disease. Those containing an acyl-tail of 16 or 18 carbons are considered deleterious due to frequent association with cardiometabolic disease. The enzyme ceramide synthase (CerS) catalyses ceramide synthesis. There are six known CerS isoforms known to exist in humans. Each isoform demonstrates specific fatty-acid substrate preference and tissue localisation. Selective pharmacological inhibition of CerS isoforms which produce deleterious ceramides, could potentially prevent cardiometabolic disease. Additionally, the subcellular localisation of ceramides and CerS is important to understanding their role in insulin resistance, but this is not yet well defined. We used subcellular fractionation paired with targeted liquid chromatography-mass spectrometry (LC-MS) to interrogate the subcellular ceramide distribution in key tissues. This study aimed to interrogate how our novel CerS inhibitor, ET2.39, alongside high fat feeding can alter these subcellular ceramides.

Male C57BL/6 mice were provided chow, high-fat diet (HFD), or HFD + a CerS inhibitor, ET2.39 (~10mg/kg/day). Cellular fractions (membrane, cytosol, nuclear and mitochondrial) from quadriceps and liver were extracted using an iodixanol gradient and ultracentrifugation. Enrichment was verified via western blotting of fraction markers. Sphingolipid profiles of fractions were analysed via targeted LC-MS which detected 62 different sphingolipid species.

Fractions demonstrated unique sphingolipid composition. Sphingolipid content was greatest in the plasma membrane and nucleus. HFD significantly increased deleterious ceramides across all cellular fractions. ET2.39 treatment significantly reduced C18 ceramides in quadriceps, with similar trends shown in cellular fractions. In conclusion, different sphingolipid distributions exist across cellular fractions, of which can altered by diet and pharmacological inhibition.

Mitochondrial phosphatidylethanolamine (PE) synthesis essentially regulates whole body lipid metabolism and metabolic health

Brunner, Sarah[1], Liebisch, Gerhard[1], Höring, Marcus[1], Janssen, Klaus-Peter[2], Burkhardt, Ralph[1], Ecker, Josef[1]

[1] University Hospital Regensburg, Germany

[2] Technical University Munich, Germany

T06

We have previously shown that the glycerophospholipid (GPL) composition of cells and tissues depends on various extrinsic factors, like diet or the gut microbiota, and intrinsic GPL synthesis and metabolism. As phosphatidylserine decarboxylase (PISD) decarboxylates phosphatidylserine (PS) to generate PE in mitochondria, it is evident that this enzyme essentially regulates GPL distribution in mitochondria–and likely also in other cellular organelles. To date, PISD is believed to be the primary source of mitochondrial PE, as other underlying mechanisms of mitochondrial PE synthesis or transport from other organelles remain poorly understood. Using stable isotope labeling to study GPL metabolism, we identified an exceptionally high PISD activity in brown adipocytes. PE levels are significantly elevated in brown adipose tissue (BAT) mitochondria compared to white adipose tissue mitochondria, as well as in BAT mitochondria isolated from mice housed at 4 °C compared to 23 °C and 30 °C. Consequently, we asked whether PISD could be related to adipose tissue browning and uncoupling protein 1 (UCP1) function. We developed a mouse model with a tamoxifen-inducible PISD knockout in UCP1-expressing cells (iBcKO-PISD). Microplate-based respirometry analyses of brown adipocytes derived from iBcKO-PISD mice revealed enhanced UCP1-mediated respiration upon β-adrenergic stimulation compared to adipocytes from wild-type animals. Several pathologies observed in brown/beige adipose tissue and liver of wild-type mice on a high-fat diet were alleviated - or even absent - in iBcKO-PISD animals following cold exposure. These included parameters related to (I) obesity–such as increased body weight and fat mass, insulin and leptin resistance, and larger lipid droplets in brown/beige adipose tissue–and (II) metabolic-associated fatty liver disease (MAFLD), including hepatic lipid accumulation, elevated plasma alanine aminotransferase (ALT) levels, and immune cell infiltration in the liver. In summary, our findings demonstrate that the activity of PISD in UCP1-expressing cells of obese animals not only improves BAT function but also regulates whole-body lipid metabolism, for example via BAT–liver crosstalk. We propose that modification of BAT GPL composition, potentially via gene editing or pharmacological targeting of PISD, dietary interventions or manipulation of the gut microbiota, could represent a promising strategy to promote metabolic health.

Plasma hexocylceramide and ether lipid enrichment as hallmark of human extreme longevity

Fernández-Bernal, Anna[1], Sol, Joaquim[1], Mota-Martorell, Natàlia[1], Mas-Bargues, Cristina[2], Obis, Èlia[1], Borrás, Consuelo[2], Pamplona, Reinald[1], **Jové, Mariona**[1]

[1] University of Lleida-Biomedical Research Institute of Lleida, Spain

[2] University of Valencia, Spain

T07

Centenarians and their offspring represent human models of successful aging, displaying exceptional longevity and reduced incidence of major age-related diseases. While previous omics studies have revealed metabolic and molecular adaptations underlying this phenotype, the contribution of lipidomics remains underexplored, particularly regarding heritable lipid signatures linked to extreme longevity.

In this study, we applied mass spectrometry-based lipidomics to analyze plasma samples from 39 centenarians, 63 centenarians' offspring, and 69 age-matched controls without familial longevity. A total of 569 lipid species were quantified, spanning multiple lipid classes. Additionally, we computed lipid indexes to assess biophysical properties such as membrane fluidity, lipid saturation, chain length, and class diversity.

Our results reveal a distinct lipidomic profile associated with familial longevity. Both centenarians and their offspring exhibit: (i) a marked enrichment in hexosylceramides, (ii) reduced levels of specific ceramides and sulfatides, (iii) a consistent increase in ether-linked phosphatidylcholines (PC) and lysophosphatidylcholines (LPC), and (iv) shifts in membrane-related indexes indicating more stable and less inflammatory lipid environments.

These findings suggest that the conversion of ceramides to glycosylated forms and the preservation of ether lipid levels may represent adaptive, heritable mechanisms that promote healthy aging. We propose that this lipidomic signature is not merely a consequence of advanced age but reflects an intrinsic longevity program that could inform novel biomarkers or therapeutic strategies for age-related decline.

This work was supported by grants from "La Caixa" Foundation (HR21-00259), the Spanish Ministry of Science, Innovation, and Universities (PID2023-152233OB-I00, PID2020-113839RB-I00, MCIN/AEI/10.13039/501100011033, co-funded by the European Regional Development Fund, "A way to build Europe"), the Diputació de Lleida (PP10605-PIRS2021 and PP10845-PIRS2023.), the Generalitat of Catalonia through the Agency for Management of University and Research Grants (2021SGR00990), the Instituto de Salud Carlos III (PI24/01431, CB16/10/00435 and AC20/00026), the European Union (GA N° 696295 of the EU Horizon 2020 Research and Innovation Program) and the Conselleria de Educació, Universitats i Ocupació of the Generalitat of Valencia (CIAICO/2022/190). IRBLleida is part of the CERCA Programme / Generalitat of Catalonia. M.J. is a Serra Hunter professor (Generalitat de Catalunya).

Beyond the gut: How the microbiome regulates whole-body lipid metabolism

Ecker, Josef[1]

[1] Functional Lipidomics and Metabolism Research, Institute of Clinical Chemistry and Laboratory Medicine, University Hospital Regensburg, Regensburg, Germany

T08

The mammalian gut harbors highly complex microbial communities, referred to as the gut microbiota or microbiome when their genomes and surrounding environmental conditions are considered. Those contribute to food processing; make non-digestible nutrients available to the host; and provide important functions in host immunity, physiology and metabolism. Using multi-omics, in vivo stable isotope tracing, dietary interventions and mechanistic investigations in mice, we could show that the gut microbiota is essential for whole body lipid metabolism: [I] the short chain fatty acid acetate produced by gut microbial degradation of fiber is metabolized to longer chain fatty acids and glycerophospholipids in the liver; and [II] intestinal absorption of dietary lipids is regulated by the microbiome. Application of antibiotics inducing a dysbiosis disturbs hepatic synthesis of lipids and their uptake from the nutrition. The metabolic interplay between the microbiome and host lipid metabolism is not only key for physiological homeostasis, but also for pathophysiology. Essential polyunsaturated fatty acids are enriched in tumors and acetate is pivotal for membrane glycerophospholipid synthesis during cell proliferation. Perturbations of the microbiome impairing short chain fatty acid generation in the intestinal lumen significantly impact tumor development in colorectal cancer and liver regeneration after partial hepatectomy.

Beyond alpha-fetoprotein: Development of a targeted lipidomics assay for accurate discrimination of HCC from other liver diseases

Merciai, Fabrizio[1], La Gioia, Danila[1], Caponigro, Vicky[1], Sommella, Eduardo[1], Campiglia, Pietro[1]

[1] University of Salerno, Italy

T09

Early and accurate diagnosis of hepatocellular carcinoma (HCC) remains a major clinical challenge, particularly in patients with chronic Hepatitis C Virus (HCV) infection and other liver disease, where standard biomarkers such as alpha-fetoprotein often lack sensitivity and specificity. At the forefront of HCC liquid biopsy of circulating tumor cells tumor DNA, open new avenue for early HCC discovery. Nevertheless, the role of lipids and metabolites is still underestimated. To address this, we implemented a two-tiered metabo-lipidomics workflow combining untargeted discovery with rapid targeted validation, aiming to identify robust circulating biomarkers capable of stratifying HCC from chronic liver diseases including HCV and Mixed Cryoglobulinemia disease (MC).

Plasma samples from 102 patients (69 HCC, 23 HCV, 10 MC) were analyzed using ultra-high-performance liquid chromatography coupled to high-resolution mass spectrometry, integrating both hydrophilic interaction chromatography (HILIC) and reverse-phase separations hyphenated with Trapped Ion Mobility Mass Spectrometry (TIMS). Untargeted analysis revealed distinct molecular patterns among groups. HCC samples were characterized by elevated short- and long-chain acylcarnitines (CARs), while medium-chain CARs remained specific to HCV. Lipidomic analysis showed a net reduction in lysophosphatidylcholines (LPCs) and a concomitant increase in phosphatidylcholines in HCC, suggesting the alteration of the Land's cycle. To translate these signatures into potential clinical utility, and provide quantitative values, we developed a fast targeted HILIC-HRMS assay employing Multiplexed Single Ion Monitoring (SIM) on a quadrupole-Orbitrap platform. The method used a 100 mm narrow-bore column with sub-2 µm particles and optimized chromatographic conditions to achieve high resolution and repeatability. A panel of 24 CARs and LPCs was quantified with excellent analytical performance: sensitivity (LOD_{avg}: 0.0558 ng mL^{-1}, LOQ_{avg}: 0.667 ng mL^{-1}), accuracy (98.75%), linearity (R^2 = 0.999), and stability (intra-day CV: 0.39%, inter-day CV: 1.46%).

The restricted panel further confirmed the diagnostic value of the identified untargeted signature. Principal Component Analysis (PCA) revealed a clear separation between HCC and HCV, which was further supported by the Partial Least Squares Discriminant Analysis (PLS-DA) model. The model demonstrated robust classification performance, achieving 96.43% accuracy on the test set, with a sensitivity of 100.00% for HCC detection and a specificity of 83.71%, supporting the clinical relevance of the identified molecular signature. This integrated approach bridges discovery and validation and paves the way for its validation on a larger scale as a novel liquid biopsy assay for HCC diagnosis.

The circulating bioenergetic lipotype as a distinctive signature in Centenarians

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T10

Introduction: Ageing and the lipidomic profile are closely related. However, the influence of longevity on bioenergetic lipids – key components present in all eukaryotic cells – hasn't been explored yet. In this study, we aimed to characterize the bioenergetic lipid profile in long-lived humans.

Methods: We conducted a targeted lipidomics analysis on plasma from healthy adults (27.9 ± 1.4 years; 14 women and 7 men), elderly (76.4 ± 0.5 years; 15 women and 7 men) and centenarians (100.8 ± 1.1 years; 19 women and 6 men). Using liquid chromatography coupled to an Agilent Technologies 6495 triple quadrupole mass spectrometer (LC-MS-QQQ), we detected and quantified 127 bioenergetic lipids, including triglycerides (TG, 44), ether TG (TG(O), 3) cholesterol esters (CE, 27), oxidized CE (oxCE, 2), diglycerides (DG, 20), acylcarnitines (AC, 14), cholesterol (CHO, 1), oxysterols (COH-OH, 10), and desmosterol esters (DE, 6). Lipidomics data was complemented with a transcriptomics analysis in peripheral blood mononuclear (PBMCs) obtained from the same individuals, which allowed for the identification of 83 genes encoding enzymes involved in bioenergetic lipid synthesis

Results: Overall, the plasma lipid composition in centenarians remained largely stable, with the exception of a selective increase in oxCE. Most differences observed were qualitative rather than quantitative, including a notable reduction in the unsaturation degree of DEs and increased in ACs. Increased CHO-OH acyl chain length and high DE diversity were also observed in centenarians. Notably, lipid adaptations in centenarians were more specific to individual lipid species rather than entire lipid classes. Additionally, transcriptomic analysis revealed that the expression of genes involved in bioenergetic lipid metabolism in centenarians resembled that of adults.

Conclusions: Globally, results suggest the existence of adaptations in lipid and energetic metabolism in long-lived humans.

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Shotgun lipidomic workflow for single cell analysis using single and multipass cyclic ion mobility

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T11

Introduction:
Technological developments have enabled the detection and identification of lipids in single cells. The ability to probe the composition of single cells allows observations of cell-cell interactions, cell states and phenotypic identification. Traditional lipidomic approaches often fail to capture the heterogeneity of lipid profiles within individual cells. Ion Mobility Spectrometry (IMS) has been adopted as an additional tool to support traditional MS-based lipidomic workflows, as this IMS adds a dimension of separation. This increases confidence through the addition of collision cross-section (CCS) values that can be compared to databases. IMS generates cleaner mass-spectral data and removes interference in complex biological samples, helping to resolve isomers. To address this limitation, we have developed a novel single-cell lipidomic workflow utilising the SELECT SERIES™ Cyclic™ IMS and LipidXplorer software. Our workflow integrates advanced ion mobility spectrometry with high-resolution mass spectrometry to achieve precise lipid identification and quantification at the single-cell level. The SELECT SERIES™ Cyclic™ IMS provides enhanced separation of lipid species based on their shape, size, and charge, allowing for the resolution of complex lipid mixtures. LipidXplorer software is employed for the automated annotation and quantification of lipid species, leveraging its robust database and algorithmic capabilities.

Sample Preparation:
Cancer cell lines were used for a proof-of-concept (POC) study. Single cells are isolated using microfluidic techniques to ensure minimal disruption to cellular integrity. Lipids are extracted using optimised protocols that preserve the native lipid composition. The extracted lipids are then introduced into the SELECT SERIES™ Cyclic™ IMS for separation and analysis using a robotic nanospray ion source using nanoelectrospray chips.

Data Acquisition:
High-resolution mass spectrometry is coupled with IMS to provide accurate mass measurements of separated lipid species. The lipids were first analysed in both positive and negative ion ESI modes on the SELECT SERIES Cyclic IMS system using High Definition MSE (HDMS^E) single pass acquisition mode to capture a comprehensive lipid profile and enable the use of the Waters Lipids CCS library for identification. Multiple passes through the mobility cell were also performed on specific ions to achieve superior resolution and separate isomers.

Data Analysis:
LipidXplorer software processes ApexRT peak-detected MassLynx™ data for identifying lipid species based on their mass-to-charge ratios (m/z) and ion mobility characteristics. The data processing pipeline facilitates the annotation of diverse lipid classes. Quantitative analysis is performed to determine the relative abundance of lipid species within single cells.

Results:
Our workflow successfully identified and quantified over approximately 300 lipid species from single cells, revealing significant heterogeneity in lipid profiles for different cell types. The cyclic IMS provided exceptional separation of lipid isomers, enhancing the accuracy of lipid identification. LipidXplorer's automated annotation streamlined data analysis, reducing the time required for lipid identification and quantification.

Discussion:
The integration of SELECT SERIES™ Cyclic™ IMS with LipidXplorer software represents a significant advancement in single-cell lipidomics. Our single-cell lipidomic workflow utilising SELECT SERIES™ Cyclic™ IMS and LipidXplorer software offers a powerful tool for the detailed analysis of lipid profiles at the single-cell level. The enhanced resolution and accuracy of lipid separation and identification enable detailed characterisation of lipid heterogeneity within individual cells. The ability to resolve and quantify diverse lipid species within individual cells provides valuable insights into cellular functions and disease mechanisms. This workflow has potential applications in various fields, including cancer research, neurobiology, and metabolic studies. Future studies will focus on expanding the lipid libraries in LipidXplorer and optimising sample preparation techniques to further enhance the workflow's capabilities.

Revealing cancer vulnerabilities in stress adaptation and ferroptosis through functional lipidomics

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T12

Cell fate decisions are profoundly shaped by lipid metabolism, which plays an essential role in both physiological and pathological contexts. Beyond their well-established roles in membrane structure and energy storage, certain low-abundance lipid species act as signaling molecules, serving as second messengers or lipokines that critically regulate processes such as programmed cell death[1,2]. Leveraging the mechanistic diversity of cytotoxic natural products, we apply functional lipidomics and multiomics to dissect lipid-mediated regulatory pathways. This strategy has proven particularly effective in investigating metabolically controlled forms of cell death, such as ferroptosis, which is driven by iron-dependent lipid peroxidation and subsequent membrane disruption. Ferroptosis has emerged as a promising therapeutic avenue for targeting EMT-driven metastatic and therapy-resistant cancers, with mesenchymal-type persister cells displaying marked sensitivity[3]. This presentation will demonstrate how functional lipidomics and multiomics enabled us to elucidate the lipid metabolic mechanisms underlying this heightened ferroptosis susceptibility[4]. Furthermore, we will present a unifying mechanism by which diverse cytotoxic stressors converge to amplify membrane peroxidation, an intrinsic feature of many cell death programs[5]. Finally, we will share initial insights from our drug discovery pipeline, which aims to exploit this sensitization mechanism to develop novel anti-cancer combination therapies.

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Neurodegeneration: Intra-Lysosomal Lipid Metabolism in the Driver's Seat

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T13

Although only 1-3% of the volume of a cell, lysosomes have emerged as critical metabolic and signaling hubs. These membrane-bound compartments degrade macromolecules and clear damaged organelles to enable cellular adaptation to various metabolic states. Lysosomal function is critical for organismal homeostasis—mutations in genes encoding lysosomal proteins cause severe human disorders known as lysosomal storage diseases and lysosomal dysfunction is implicated in age-associated diseases including cancer, metabolic syndrome and neurodegeneration. However, the biochemical basis of the lysosomal dysfunction and how it leads to human diseases remain to be discovered.

In my talk, I will present our research leveraging lipidomics tools to elucidate the role of the lysosome in neurodegeneration through decoding lysosomal gene function. This work led to the discovery of novel lipid synthesis, degradation, and transport pathways whose loss causes neurodegenerative diseases, thus providing a foundation for potential therapeutic interventions.

Lipid Metabolic Defects Underlie Parkinson's Disease Pathology

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T14

Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by dopaminergic neuron loss and accumulation of pathological alpha-synuclein (ASYN) and lipids forming Lewy pathology. Lipid metabolic defects are increasingly recognized as critical contributors to PD pathogenesis, influencing ASYN aggregation, mitochondrial oxidative stress, and impairing autophagic and lysosomal pathways essential for clearing pathological ASYN and maintaining cellular homeostasis. Here, we perform LC-MS/MS based lipidomics across brain and fluid biospecimens from PD and related patient cohorts to elucidate the underlying correlations between altered lipids at the species level to ASYN pathology across brain regions of interest, patient genetic status and patient subtypes stratified by progress to dementia.

Brain concentrations of glucosylsphingosine 18:1;O2 (GlcSph), a substrate for lysosomal glucocerebrosidase, were elevated in GBA1 mutation-carrying and idiopathic subgroups and were highly correlated with progression to dementia with greatest associations observed in cingulate cortical regions exhibiting a high degree of ASYN pathology. Lipidomic analyses performed in plasma and cerebral spinal fluid (CSF) specimens obtained from sporadic and GBA and LRRK2 mutation carrying patient subgroups. GlcSph fluid elevation was limited to plasma from GBA mutation carriers only suggesting limited utility for use a clinical biomarker. However, analyses of the full lipidomic data sets acquired from plasma and CSF biospecimens identified fluid lipid alterations associated with PD independent of mutation status.

Collectively, these findings highlight common lipid metabolic defects across PD subgroups and underscore the pivotal role of lysosomal lipid dysregulation in ASYN pathology and dementia progression in PD, offering new insights into disease mechanisms and potential therapeutic targets for future development.

Pathway strength score computation and utilization as a lipid metabolic index to assess novel transglycosidase function in GBA1-PD patients

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T15 PRIZE

Lipidomic signatures in biofluids are under assessment as potential prognostic and monitoring biomarkers. The metabolic interpretation of these steady-state signatures is often challenging due to (1) the diversity and complexity of the lipidome, (2) the interconnection of lipid metabolism wherein the level of each lipid molecule is the result of multiple enzymatic reactions, and thus (3) statistical changes (or lack thereof) in lipid abundances is not simply a direct result of a mutation but rather a snapshot of a change of state across the entire metabolic system. We present here a strategy to derive a clinically relevant metabolic index of this change-in-state from mass spectrometry-based quantification of lipid abundances in different matrices.

Variants in GBA1 represent the largest share of genetic Parkinson's Disease (PD) burden worldwide. GBA1 encodes the lysosomal beta-glucocerebrosidase enzyme (GBA1-GCase) that releases the glucose headgroup from glucosylceramides (β-GlcCers) and glucosylsphingosine (β-GlcSph). We have pursued a second function of GBA1 and found that the enzyme does not only release glucose from β-GlcCers/GlcSph but also catalyzes the reversible transglycosylation (GBA1-TGase) of not only glucose but also its isomer galactose from β-GlcCers and β-GalCers to cholesterol producing glucosylcholesterol (β-GlcChol) and galactosylcholesterol (β-GalChol) respectively. The bidirectionality of this reaction is regulated by the lysosomal concentration of cholesterol. We present here our targeted lipidomic pipeline enhanced by differential mobility spectrometry which detects and quantifies β-GlcChol and β-GalChol in human plasma. Our established lipidomic pipeline consists of nanobore reversed-phase liquid chromatography-electrospray ionization-tandem mass spectrometry (RPLC-ESI-MS/MS) followed by information-dependent-acquisition of enhanced product ion scan (IDA-EPI) for accurate quantification and confident identification of the lipid substrates and products in the GBA1-TGase pathway. We also present a novel algorithm which utilizes lipidomic measurements to calculate the strength of targeted metabolic pathway. This pathway strength is direction specific and provides a lipid metabolic index that pinpoints where changes-of-state have occurred across the broader metabolic network. We report here our quantification of β-GlcCers and β-GalCers, as well as β-GlcChol and β-GalChol and show that the expression of GBA1-PD variants alters GBA1's transferase activity (a novel gain of GBA1 enzymatic function) in human plasma.

Region-Specific Lipid Remodeling in Alzheimer's Disease: A Spotlight on White Matte

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T16

This study presents a comparative lipidomic analysis of cerebral gray matter (GM) and white matter (WM) during the progression of sporadic Alzheimer's disease (sAD), employing untargeted LC-MS/MS and gas chromatography. Post-mortem samples from 35 individuals including 10 healthy controls and 25 Braak stages I–VI focused on frontal cortex Area 8 (GM) and the centrum semiovale (WM). The investigation aimed to unravel region-specific lipidomic signatures in healthy and affected brain tissue, and their functional implications in neurodegeneration. Lipidomic profiling encompassed fatty acid composition, peroxidation indices, and molecular species analysis by a non-targeted approach. Data validation integrated RT-qPCR for lipid homeostasis genes and immunohistochemistry (IHC) for associated proteins. Statistic analyses resolved lipid dynamics across Braak stages, emphasizing spatial and temporal lipid remodeling.

White matter exhibited a distinct adaptive lipid phenotype in healthy adults characterized by increased unsaturation (+32% in WM compared to GM) basically due to increased amount of monounsaturated fatty acids (MUFA: 47.8 vs. 28.6 in GM, +67%) and decreased levels of polyunsaturated fatty acids, especially omega-3 (PUFA n-3: 3.77 vs. 8.33 in GM, -55 %), and saturated fatty acids (SFA: 40.4 vs. 54.7 in GM, -26%). In AD progression according to Braak stages, we observed an increase in double bond index in GM (DBI, Spearman's Rho correlation p = 0.048) and a trend toward an increase in the peroxidation index (PI, Spearman's Rho correlation p = 0.067), making the fatty acid profile in GM more prone and sensitive to oxidation as the disease advance. Meanwhile, changes in these indexes in WM were not significant, thus maintaining its resistant profile towards oxidation.

Nevertheless, the whole lipidome of WM was more affected than that of GM. Specifically, advanced stages of sAD correlated with decreased levels of ether lipids in WM, particularly alkyl and alkenyl glycerophosphocholines (PC) and glycerophopshoethanolamines (PE). Other lipids also decreased in WM during sAD progression, including diglycerides and triglycerides containing monounsaturated fatty acids, ceramides, and cerebrosides. In GM the differences were less pronounced, with decreased levels of triglycerides in advanced stages, as well as reductions in one ether PC and one fatty acid ester (FAHFA). Interestingly, many of the species that were significantly different between WM and GM in control subjects, were later affected during sAD progression.

These results were validated by RT-qPCR and IHC, which revealed alterations linked to sAD progression in lipid homeostasis gene expression and regional protein distribution. These changes affected WM more extensively than GM and indicated a reshaping of PUFA and FAHFA biosynthesis, fatty acid peroxisomal import and β-oxidation, and peroxisomal biogenesis orchestrated by PPARG and PGC1α.

The pronounced WM lipid dysregulation suggests a pivotal role in axonal disconnection and sAD progression. Loss of plasmalogens, ceramides and cerebrosides could impair glial signaling and myelin integrity, while lipid oxidation markers reflect sustained oxidative stress. These mechanisms may precede classical neuropathological hallmarks, positioning WM lipidomics as a potential biomarker source and therapeutic target. Gene expression and protein data further linked lipid homeostasis adaptations to each region, highlighting the importance of WM changes in the myelin sheath and oligodendrocyte lineage cells in the pathology of sAD.

The metabolic cost of the lipidomic network-level response to ASAH1 mutation differentiates and provides new therapeutic options for Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy from Farber's Disease

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T17

Spinal Muscular Atrophy with Progressive Myoclonic Epilepsy (SMA-PME; OMIM 159950) and Farber Lipogranulomatosis (FL; OMIM 228000) are rare, allelic, fatal, autosomal-recessive disorders caused by pathogenic variants of the N-acylsphingosine amidohydrolase 1 gene (ASAH1). ASAH1 encodes for acid ceramidase (aCDase, EC 3.5.1.23), one of five ceramidases that hydrolyze ceramides and dihydroceramides to a free fatty acid and a long chain sphingoid base. SMA-PME is a mid-childhood onset neurological disorder characterized by progressive weakness in the proximal muscles of the limbs followed by epileptogenesis. By contrast, FL is an early onset peripheral disorder defined by painful joint deformation, development of sub-cutaneous nodules, a hoarse cry and failure to thrive. All ASAH1 variants result in a loss of function, leading to the accumulation of ceramides (d18:1) in the lysosomes of patient cells in both disorders. It is not known how comparable loss of function results in two different diseases. Here, we used LC-ESI-DMS-MS/MS to fully elucidate the sphingolipid and deoxysphingolipid metabolic networks in patient fibroblasts, induced pluripotent stem cell-derived patient-derived neurons, and two novel CRISPR-Cas9 "knock-in" mouse models carrying either homozygous (FL) or compound heterozygous (SMA-PME) mutations. Differential ion mobility spectrometry was used to quantify glucosyl and galactosyl sphingolipid epimers at the molecular level enabling us to profile the complete repertoire of sphingolipids with d18:1, d18:0, and t18:0 sphingoid bases, including ceramides glucosylceramides, glucosylsphingosine, galactosylceramides, galactosphingosine, sphingomyelins, lactosylceramides, and globotriaosylceramides. Using novel lipidomic pathway strength analyses, metabolic enrichment analyses, and subcellular organelle lipidomic assessments, we present here the network level metabolic changes that discriminate between FL and SMA-PME. We find that the two diseases are defined by a different metabolic response to rising ceramide levels and that it is the "metabolic cost" of these changes that associates with disease presentation by differentially impairing lysosomal, mitochondrial, and endoplasmic reticulum function. Comparing enzyme replacement with metabolic intervention using the histone deacetylase inhibitor SAHA and the uricosuric drug benzbromarone, we show that pharmacologically alleviating the metabolic cost of these changes in addition to substrate reduction provides new treatment avenues for both disorders.

LC-MS based analysis of phosphoinositides (PIPs) in metabolic, cardiovascular and degenerative disease

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T18

Phosphorylated forms of phosphatidylinositol (PI) called phosphatidylinositol phosphates (PIPs), act as messengers that play important roles in lipid signaling, cell surface signaling, and membrane trafficking. Therefore, altered or dysregulated levels of PIPs provide first hints for their involvement in cardiovascular, neurodegenerative, cancer and metabolic disorders e.g., obesity and diabetes. To synthesize PIPs in cells, the inositol ring of the PI is enzymatically phosphorylated by different kinases on the 3rd, 4th, and 5th hydroxyl groups generating seven different mono-, bi- and tri-phosphorylated analytes of which some are isomers. Despite the importance of PIPs, only very few LC-MS based analytical methods that allows separation of seven regioisomeric PIPs with different fatty acyl chains and degrees of phosphorylation have been described. Recently, two groups [Peng Li 2021; Morioka Shin 2022] showed that chemical derivatization using trimethylsilyldiazomethane (TMSD) of the extracted lipids under acidic conditions and subsequent analysis on chiral polysaccharide-based stationary phase column chromatography coupled to high-resolution mass spectrometry (HR-MS) enabled both enrichment and detection of the endogenous PIPs in the biological samples.

To implement and improve this combined approach, we employed CHIRALPAK® IB-U (Daicel, Japan) column installed on a UHPLC system (Vanquish Horizon) coupled to Exploris 240 mass spectrometer (both Thermo Scientific). We initially tested the separation efficiency with a complete set of seven PIP internal standards (IS) i.e., 17:0-20:4 PI(3,4,5)P1/2/3 (Avanti Polar Lipids) that were subjected to TMSD derivatization. After few optimizations of the LC method (i.e., flow rate, gradient type/length, column temperature), we reproduced and improved the results from the previously published work. Next, using the same set of IS, we generated calibration curves ranging from 125 fmol to 100 pmol for each PIP regioisomers and conducted recovery experiments (HeLa cells as background) with our optimized sample preparation protocol and chiral LC-MS platform. Furthermore, we used the same methodology to detect endogenous PIPs in H9c2 cells (immortalized myoblasts derived from embryonic rat hearts) that are used particularly for studying cardiac physiology and pathophysiology.

A Highly Sensitive and Selective Targeted Method for Single Cell Lipidomics

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T19

Lipids play critical roles in multiple biological processes and their heterogeneous distribution among cells has significant biological implications. Single-cell analysis is hence critical to understand cell-to-cell heterogeneity that would not be revealed in bulk analysis. Here we develop a targeted method on the Stellar mass spectrometer, with nanoflow chromatography, to both increase the detection sensitivity, to what is required for a single cell, and achieve the selectivity and specificity of each lipid beyond the species level.

Lipids were extracted from one hundred RK13 and Vero cells and separated on a 75-micron RP column connected to a Vanquish Neo UHPLC system. Data was acquired on the Orbitrap Ascend Tribrid MS using an untargeted multi-stage strategy for fragmentation with HCD and CID both utilized for MS2 followed by CID based MS3 fragmentation of specific product ions generated by the neutral loss of fatty acyl chains. This template for lipid analysis provides individual fatty acids for multiple lipid classes including co-eluting TGs. CD 3.4 with the LipidSearch node was used for data processing and a targeted list of around 900 confidently annotated lipid species, combining both the cell lines, was generated. A targeted method for the 900 confidently annotated lipids was created using Parallel reaction monitoring (PRM) on the Stellar MS and the same activation types, HCD and CID MS/MS and MS3 capabilities as on the discovery MS. PRM of the targets significantly enhances specificity and sensitivity for isomeric lipids and structurally similar species.

RK13 and Vero single cells were collected in a 96 well plate, extracted using isopropanol and analyzed with polarity switching using the targeted method developed on Stellar. Major differences were seen between the two cell types as well as between the cells in different conditions.

Novel Aspects
A targeted method for single cell lipidomics was developed to make this application more accessible to the broad research community.

Disentangling Complex Brain Lipid Phenotypes in Parkinson's Disease

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T20

Mutations in GBA1, encoding lysosomal glucocerebrosidase (GCase), are a risk factor for Parkinson's disease. Reduced GCase function results in accumulation of glycosphingolipid substrates and imbalances in downstream ganglioside metabolites. Here we performed in-depth characterization and region-specific localization of brain gangliosides in a GBA mutant mouse model using next-generation ion mobility instrumentation and mass spectrometry imaging. We identify dark corners of the ganglioside lipidome and identify their region-specific localization in brain. Ongoing work evaluates how well the lipid findings translates to PD patient brain specimens. Our work opens new avenues to tackle the dysfunctional glycolipid metabolism accompanying neurodegeneration.

Lipidomics at the Single-Cell Level: Workflow Optimisation for Live Cell Analysis

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T21 PRIZE

We present an optimised workflow for single-cell lipidomics using capillary sampling and LC-MS to profile live pancreatic cancer cells. Systematic evaluation of sampling variables—including capillary tip type, aspiration volume, and storage conditions—revealed critical factors influencing lipid detection sensitivity. Both manual and automated sampling methods yielded comparable lipid profiles when key parameters were controlled. Our findings underscore the importance of appropriate blank correction and methodological consistency, laying the groundwork for robust single-cell lipidomics applicable to biomarker discovery and metabolic research.

Single-cell lipidomics is a rapidly evolving field that enables the profiling of lipid species from individual cells, offering new insights into cell-to-cell heterogeneity in health and disease. However, the technical challenges associated with sampling and analysing lipids from live single cells remain a major barrier to widespread adoption.

Here, we present an optimised workflow for live single-cell lipidomics using capillary microsampling combined with liquid chromatography–mass spectrometry (LC-MS). We systematically evaluated critical variables influencing sampling success and lipid detection, including capillary tip diameter, aspiration volume, sampling buffer composition, and the position of the cell during aspiration.

The workflow was validated using both manual and automated sampling approaches on adherent pancreatic cancer cells, with both methods yielding comparable lipid profiles when key parameters were controlled. To enhance reproducibility and data quality, we incorporated rigorous blank correction and quality control strategies.

This optimised method improves the sensitivity and robustness of live-cell lipidomics while remaining low-cost and accessible, requiring no specialised microfluidic chips or cell lysis. Our findings demonstrate that careful optimisation of sampling parameters is essential for reliable single-cell lipid profiling.

This approach lays the groundwork for high-confidence, live-cell lipidomics and offers broad applicability for studying lipid metabolism in cancer, stem cells, and other systems where single-cell resolution is critical.

Identification of double bond location in unsaturated free fatty acids of human sebum

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T22 PRIZE

Skin surface lipids (SSLs) consist of epidermal and sebaceous lipids. Free fatty acids (FFAs), in particular monounsaturated FAs (MUFAs) enriching sebum, are particularly abundant in seborrheic body areas, where sebaceous glands (SGs) present in higher number. Sebum FFAs exhibit unique characteristics, including a prevalence of C14-C18 chain lengths, terminal branching, and a double bond at the Δ6 position. Notably, over one-third of the sebaceous FAs contain a single double bond. Alterations in the synthesis of MUFAs can lead to non-physiological composition of sebum, potentially contributing to the pathogenesis of inflammatory skin conditions such as acne vulgaris, rosacea, psoriasis, and seborrheic dermatitis. A comprehensive characterization of unbound MUFAs, based on their different isomeric forms distinguished by the position of the carbon-carbon double bond, has not been achieved in human sebum. Therefore, we aimed to develop an analytical strategy to differentiate the various carbon-carbon double bond positions in FFAs. Our methodology combines the Paternò-Büchi reaction, utilizing 2-acetylpyridine, with liquid chromatography and tandem mass spectrometry (LC-MS/MS). This strategy was initially optimized on standard FFAs and subsequently applied to human sebum.

The method enabled the characterization of seventeen MUFAs with C14-C18 chain lengths and two C18 polyunsaturated FAs, namely linoleic and sebaleic acid. The predominance of sapienic acid (C16:1n-10) and its elongated congener (C18:1n-10) among MUFAs indicated the dominance of the Δ6 desaturation pathway catalyzed by the FADS2 enzyme in human SG. The quantitative assay was applied to sebum sampled from male and female healthy donors, proving comparable abundance of MUFAs and PUFAs.

Investigating the analytical response of triglycerides by flow-injection-analysis high-resolution mass spectrometry

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T23

Triglycerides (TG) play a key role in energy and fat storage; consequently, they are associated with health risks such as metabolic syndrome.

In this study, we applied flow-injection-analysis high-resolution mass spectrometry (FIA-FTMS) to systematically investigate the analytical response of TG species. Our results demonstrated that the TG response is linked to the polarity of the species (i.e. the number of acyl carbons and double bonds) and the polarity of the infusion solvent. TG ion aggregate formation is probably the primary factor causing these response effects, indicating that the TG response depends on its concentration in the infusates.

To translate these findings to biological samples, we analyzed adipose tissue lipid extracts and optimized measurement conditions, including solvent system, infusate concentration, and the selection of an appropriate internal standard. These optimizations improved the accuracy and reproducibility of quantification, making lipidomic profiling of neutral lipid-rich samples, such as adipose tissue, more reliable.

Technical recommendation for the quantitative analysis of oxylipins and new ideas for targeting them in oxidized phospholipids

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T24

Polyunsaturated fatty acids (PUFA) are prone to oxidation. Both enzymatic as well as non-enzymatic processes can lead to a large number of oxidation products, including eicosanoids and other oxylipins.

Several oxylipins are potent lipid mediators, playing a key role in the regulation of e.g., inflammation, vascular tone and blood coagulation. Oxylipins present unique analytical challenges, including low abundance, rapid degradation and artificial formation, the presence of many closely eluting isomers, and similar fragmentation patterns. Based on an initiative of the ILS oxylipin interest group, technical recommendations for targeted liquid chromatography tandem mass spectrometry (LC-MS/MS) have been developed, which enable a more accurate and reproducible quantification of oxylipins (doi: 10.1126/scisignal.adw1245). These are summarized in the first part of the talk.

Oxylipins are quantified by LC-MS/MS in their free, i.e. non-esterified form. However, oxylipins do not only occur non-esterified, but also bound in different kinds of fatty acid-bearing lipids. In fact, 90% of the hydroxy- and epoxy-PUFA present in human plasma or cells are esterified and largely occur in phospholipids (PL). These oxidized phospholipids (oxPL) are formed by incorporation of oxylipins into lysoPL, e.g. catalyzed by the enzymes of the Lands cycle, or by direct oxidation of PL.

Esterified oxylipins are commonly analyzed indirectly following hydrolysis of the lipids and quantification of the resulting non-esterified oxylipins. This allows a sensitive, accurate and precise quantification. However, only the sum of each esterified oxylipin is quantified and the information in which lipid class/species and sn-position oxylipins are bound is lost.

Non-targeted lipidomics allows the parallel semi-quantification of lipids, and we recently demonstrated that more than 400 oxPL species bearing oxylipins could be detected in human cells (doi: 10.1016/j.aca.2024.343139). Based on that, we developed a targeted LC-MS/MS method for PL bearing hydroxy-PUFA, allowing the selective quantitative measurement of oxPL species (https://doi.org/10.1016/j.jlr.2025.100841).

A less specific, but more sensitive orthogonal approach for the lipid class selective analysis of esterified oxylipins is a solid-phase extraction-based fractionation of lipid classes prior to targeted oxylipin analysis.

In the talk, the strengths and weaknesses of the three approaches for the analysis of oxPL are discussed. All methodologies are applied on human plasma and HEK cells, demonstrating a distinct distribution of hydroxy-PUFA from different precursors in oxPL: In supplemented as well as 15-LOX2 overexpressing cells, 15 HETE and 15 HEPE were found in PI, while 17-HDHA was in PE and 13-HODE in PC. The distinct incorporation of 15-LOX-2 products from different PUFA into PL might contribute to the biological effect of these oxylipins and their precursor fatty acids.

Targeted multimodal analyses to spatially map octadecanoid metabolism in the lung

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T25

INTRODUCTION

Asthma is characterized by chronic inflammation in the lung that is mediated by a combination of immune and structural cells. These different cell populations synthesize multiple metabolites that impact asthma pathogenesis via controlled regulation of inflammatory processes. There is a need for methodology to determine the spatial heterogeneity of metabolism and the associated cell types in the lung to increase our understanding of the etiology of respiratory diseases.

METHODS

We present here a multi-modal method to perform combined targeted mass spectrometry imaging (MSI) and targeted spatial transcriptomics in human lung tissue. Distal lung explants were obtained post-excision from patients undergoing lobectomies and challenged ex vivo (e.g., anti-IgE, IL-13). Metabolite distribution was mapped using desorption electrospray ionization (DESI) with targeted multiple-reaction-monitoring (MRM) data acquisition using a DESI-XS ionization source coupled to a Xevo-TQ Absolute triple quadrupole (DESI-MRM). Metabolite data were benchmarked using reversed-phase liquid chromatography coupled to a triple quadrupole mass spectrometry (LC-MS/MS). Single Cell Resolution IN Situ Hybridization On Tissues (SCRINSHOT) was subsequently conducted on the same cryosection to map RNA and profile cellular niches. To process this new multimodal data type, we implemented an open-source workflow to process metabolite ion images from DESI-MRM data (quantMSImageR, in-house developed R package, https://github.com/targeted-lipidomics/quantMSImageR), annotate anatomical and cellular regions (CellProfiler) and map the annotations between ion and microscope images of different spatial resolutions and acquisition areas (napari based tools). The TissUUmaps open-source tool (https://tissuumaps.github.io) was used to combine targeted imaging modalities with histological staining (e.g., hematoxylin and eosin, H&E, staining) enabling integrated analyses of all imaging modalities.

RESULTS

This multi-modal workflow was able to sequentially co-register metabolites and RNA distributions from the same cryosection, enabling visualization of metabolic and cellular responses to ex vivo challenges. Results were integrated with histological staining to correlate metabolite and cellular patterns with physiological responses. This targeted DESI-MRM approach was able to image octadecanoid metabolism in human lung tissue in response to ex vivo challenge.

CONCLUSIONS

This multimodal workflow utilizes targeted spatial mapping of metabolism in the lung by combining DESI MRM-based MSI with SCRINSHOT spatial transcriptomics. The use of targeted imaging approaches decreases the cost of the analysis, enabling the inclusion of multiple replicates while simultaneously increasing both the selectivity and sensitivity. This integration enables routine visualization of the distribution of metabolites and associated cell type in the lung, enabling novel insights into biochemical processes in the airways.

Design-Optimized Extraction Unlocks Comprehensive Lipidome of Fucus vesiculosus

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T26 PRIZE

The valorization of brown macroalgae lipids is hindered by their rigid cell walls and the lack of standardized extraction protocols that balance efficiency with comprehensive lipidome coverage. We therefore applied a four-factor full-factorial Design of Experiments (DoE) to quantify how homogenisation time, ultrasonication, extraction-cycle number and water addition steer Bligh & Dyer (BD) extraction of edible Fucus vesiculosus. Lipid extracts were profiled by untargeted LC–QTOF-MS/MS.

Synergistic mechanical disruption (5 min homogenization + 15 min ultrasonication) with two extraction cycles (MeOH:CH_3Cl 2:1, v/v) and 2.3 mL water achieved 3.44% lipid yield (dry weight) and identified 270 molecular species across ten classes, including polar glycolipids (e.g., MGDG, DGDG), sulfolipids (SQDG), betaine lipids (DGTSA), and neutral triacylglycerols (TG). Notably, diacylglyceryl glucuronide (DGGA)—linked to phosphate starvation in microalgae—and ether-linked digalactosyldiacylglycerol (EtherDGDG), previously reported only in green algae, were first characterized in Fucus, expanding the known structural diversity of macroalgal lipids.

A comparative analysis with the Folch method, conducted under identical pretreatment conditions, demonstrated a clear polarity-driven divergence. Folch yielded marginally higher lipid mass (3.63%) but preferentially enriched neutral lipids (e.g., TG), whereas the optimized BD protocol preserved 30 additional polar species, including sulfolipids (SQDG) and betaine lipids (DGTSA). Multivariate analysis (PCA) confirmed that solvent polarity—rather than mechanical stress—drives these class-level differences.

In summary, this study demonstrates that precise control of pretreatment and solvent composition enables access to a broad chemical space of Fucus vesiculosus lipids. The resulting high-resolution lipid map provides a robust platform for nutritional profiling and targeted isolation of bioactive compounds. To enhance reproducibility, future studies should adopt standardized reporting frameworks (e.g., detailed extraction parameters and QC metrics), ensuring cross-study comparability in marine lipid research.

Quantification of Lipids in Human Plasma using MS-DIAL: A Comparison of HILIC and RP Chromatography in Untargeted LC-HRMS Lipidomics

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T27 PRIZE

Lipidomics plays a crucial role in elucidating the molecular mechanisms in the context of diseases. Accurate and optimized quantification of lipids in human plasma is essential to monitor potential biomarkers or therapeutic interventions. However, the diversity and complexity of lipids in biological samples pose analytical challenges, particularly in terms of method selection, standardization and quantification.

Here, we compare two complementary separation techniques for the analysis of lipids in human plasma: reversed phase (RP) chromatography and hydrophilic interaction liquid chromatography (HILIC). Lipids are detected using untargeted high-resolution mass spectrometry (HRMS) [Anal Bioanal Chem, 2024, 416, 925] and data evaluation is carried out with the open-source software MS-DIAL [Nat Methods, 2015, 12, 523]. While HILIC separates lipids based on headgroup polarity, enabling class-based elution, RP chromatography primarily separates by acyl chain hydrophobicity, e.g. allowing for improved separation of lipid isomers. Both approaches utilize data-dependent MS/MS acquisition and are evaluated for their performance in untargeted lipid quantification using one internal standard per lipid class.

The comparative evaluation of both separation techniques reveals systematic differences in terms of matrix effects, co-elution and quantitative results. HILIC-HRMS offers clear advantages for class-based quantification of lipids in human plasma such as phosphatidylcholines or sphingomyelins, whereas RP-HRMS provides better separation of neutral lipids and lipid isomers. The results underline the importance of chromatographic separation in untargeted lipidomics.

To evaluate the quantitative performance of both methods, the results were compared with those obtained using a charged aerosol detector (CAD). In this reference method, the absolute concentrations of lipid classes following HILIC separation were determined independently of matrix-related ionization effects through external calibrations. These results were then compared with the concentrations of lipid classes determined by LC-HRMS demonstrating that HILIC- and RP-HRMS based quantification strategies lead to reasonable results. This supports good quantification accuracy of LC-HRMS using MS-DIAL.

Correlating abundance levels of lipid mediators with tissue proteomics data suggests pro-resolving functions of polyunsaturated fatty acids

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T28

Lipid mediators including free fatty acids, oxylipins, lysolipids, endocannabinoids and bile acids may act as autacoids mediating specific functions. However, more systematic research is required to better understand the physiologic implications of these molecules. Here we used an integrated mass spectrometry-based lipidomics and proteomics approach to determine expression patterns across plasma and twelve different organs and tissues in sheep (ovis aries). We assumed that the abundance of lipid mediators in tissues may correlate with the exertion of physiologic functions. The untargeted analysis method robustly identified 206 different molecules, including the previously undefined oxylipin 4-hydroxy-eicosatetraenoic acid (4-HETE), the identity of which was verified by chemical synthesis. Some lipid mediators were found to display tissue-specific expression patterns. Using the proteomics data, functional GO terms were assigned to each organ and tissue. Co-expression analysis and clustering defined 18 distinct modules with correlating proteins and lipids. Some modules were found enriched with proteins associated with specific GO terms, which was used for functional characterization of these modules. Some obviously meaningful correlations observed in these modules demonstrated this approach to work. Remarkably, high levels of polyunsaturated fatty acids (PUFAs) were found to correlate with high levels of endoplasmic reticulum and ribosomal proteins. Low abundance of these PUFAs in cartilage, muscle and tendon tissues was associated with low levels of pro-resolving mediators and low protein turnover, which may relate to their susceptibility for chronic inflammation. The present data thus suggest that PUFA-derived lipid mediators correlate with protein synthesis, turnover and regenerative capacity.

LC=CL: A software tool for unmasking C=C positions in complex lipids by routine LC-MS/MS lipidomics

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T29

Accurate identification of carbon–carbon double bond (C=C) positions in complex lipids is critical for understanding physiological and pathological lipid functions, yet remains largely inaccessible in high-throughput settings due to limitations in current analytical approaches. Traditional methods for localizing C=C bonds rely on specialized instrumentation or chemical derivatization techniques that often reduce sensitivity, restrict compatibility with standard workflows, and complicate quantification. Here, we demonstrate that terminal C=C positions (ω-positions) for each fatty acyl (FA) in complex lipids are inherently encoded in their elution profile in routine reverse-phase liquid chromatography (RPLC). Based on this framework we developed the LDA C=C Localizer (LC=CL). LC=CL is a robust, automated computational tool that enables FA-specific C=C position assignment directly from routine RPLC-tandem mass spectrometry (RPLC-MS/MS) data, without requiring modifications to instrumentation or sample preparation.

LC=CL is integrated into the widely adopted open-source Lipid Data Analyzer (LDA) software, ensuring compatibility with any MS/MS acquisition method. This enables researchers to readily access high-resolution C=C position information in large-scale lipidomics studies. The tool is supported by a comprehensive RT database (RT-DB) encompassing more than 2,400 ω-position-resolved complex lipid species, including 1,145 experimentally verified novel compounds. This RT-DB is designed to be easily extendable by the broader scientific community. LC=CL employs a machine learning algorithm that maps the elution profile contained in the RT-DB to various chromatographic conditions, with an average prediction accuracy within only a few seconds of experimental values.

The method was benchmarked against established C=C localization techniques, including electron-activated dissociation (EAD), Paternò-Büchi derivatization (PB), and ozone-induced dissociation (OzID), which demonstrated high agreement of ω-position assignments for identified molecular species. LC=CL was shown to have the highest sensitivity and is, unlike e.g. EAD, capable of identifying lipid C=C positions across both positive and negative ionization modes. These factors significantly enhance lipidome coverage and establish LC=CL as a powerful untargeted global screening approach to identify C=C isomers of interest. Notably, LC=CL is compatible with any ion activation method, including EAD, PB, OzID, etc.

To demonstrate the power and versatility of LC=CL, we reanalyzed a previously published lipidomics dataset and uncovered previously unrecognized ω-position specificity of cytosolic phospholipase A_2 ($cPLA_2$), a key enzyme involved in inflammatory lipid mediator biosynthesis. Specifically, LC=CL enabled the identification of mead acid (MA, 20:3(n-9)) in the sn-2 position of complex lipids as a selective $cPLA_2$ substrate alongside the canonical arachidonic acid (AA, 20:4(n-6)), a discovery that would have remained obscured in the absence of precise ω-position resolution. In contrast, complex lipids containing other ω-position isomers of 20:3, including 20:3(n-6) and 20:3(n-7) were not utilized by $cPLA_2$. These findings were confirmed using a targeted EAD approach combined with FA precursor supplementation to increase the abundance of relevant lipid species. This highlights the biological significance of distinguishing between ω-position isomers in functional lipidomics and reinforces the necessity for structure-resolved lipid analysis at the systems level.

In conclusion, LC=CL offers a systematic and broadly applicable approach for the FA-specific localization of C=C in complex lipids, using only standard RPLC-MS/MS data. Thereby, LC=CL facilitates access to structural information that has traditionally been difficult to obtain in high-throughput lipidomics workflows. This capability enables detailed investigations into lipid function, enzymatic selectivity, and metabolic regulation. Accordingly, ω-position information made readily accessible by LC=CL will advance the field toward more authentic and accurate insights from a metabolomic perspective.

MetaboLights - Open Access Metabolomics and Lipidomics Data Repository

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T30

Open data practices play a vital role in advancing metabolomics, enabling experimental reproducibility, speeding up scientific discoveries and supporting the creation and widespread use of standardized protocols, community guidelines, and quality control practices that enhance data interoperability.

MetaboLights is a global cross-species and cross-technique database for metabolomics and lipidomics studies, including raw experimental data and the associated metadata, covering also metabolite structures and their reference spectra. The adoption of standards (data/metadata) and the usage of ontologies with expert curation adds value and enables the reanalysis of studies by the community as well as compliance to FAIR (findability, accessibility, interoperability and reusability) data principles.
The repository accepts individual and programmatic submissions, accommodating contributions from companies, Phenome Centres, and other large-scale laboratories. The team collaborates with international repository partners and contributes to the development of tools that facilitate the discovery and integration of metabolomics and multi-omics research, as well as standardisation efforts (mQACC, OORF and HUPO-PSI).

MetaboLights also engages in outreach activities—offering courses and webinars to introduce metabolomics to the public and researchers from other disciplines and organizing workshops at international conferences to foster broader community involvement.

New to 2025, full MetaboLights accession numbers are assigned only after the data submission is finalised, ensuring the completeness and compliance of the submitted datasets, standardising further all new available studies. The team has developed the new study Validation Framework v2 and new minimum submission requirements, to scale the submission process, improving reporting and speeding up data release. A major workflow improvement now allows submitters to make their studies public independently, without the need to contact the MetaboLights team, significantly reducing the time to public availability. Manual curation is now reserved for specific cases, further streamlining the overall process.

Finally, MetaboLights, Metabolomics Workbench and GNPS/MassIVE, have started working to establish 'Metabolomics Hub', a global, unified and FAIR-compliant metabolomics consortium, to standardise open data practices in metabolomics and enable and increase the exchange, discovery, and reuse of metabolomics data.

Metabolomics Hub: International Data Exchange and Data Representation Standards for Metabolomics & Lipidomics

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T31

With the growth of public metabolomics & lipidomics datasets worldwide, there is an urgent need for standardized, interoperable repositories that promote international collaboration. A novel global consortium designed to standardise open data practices in the field, by creating a unified, FAIR (Findable, Accessible, Interoperable, and Reusable)-compliant infrastructure for the exchange, discovery, and reuse of metabolomics data, has been recently funded by the Chan Zuckerberg Initiative (CZI).

Building on the successful models of ProteomeXchange and the International Nucleotide Sequence Database Collaboration (INSDC), the consortium brings together major repositories — MetaboLights, Metabolomics Workbench, and GNPS/MassIVE as the starting point—and partners with researchers, funders, journals, and technology vendors.

This initiative will implement a harmonized data model for datasets in all data repositories included in the Consortium, develop open-source tools and software, and promote community adoption of open standard data formats such as mzTab-M and Universal Spectrum Identifiers (USIs) maintained by HUPO-PSI. Moreover, a centralized web portal will be developed to provide unified access to standardized public datasets, enabling discoverability of open datasets, which will enable large-scale meta-analyses and improvements in experimental reproducibility. This collaborative infrastructure lays also the groundwork for data-driven discovery and data integration with other omics.

New frontiers in shotgun lipidomics

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T32

In shotgun lipidomics direct infusion of total lipid extracts into a high-resolution mass spectrometer produces highly convoluted FT MS and FT MS/MS spectra and it seems counterintuitive how shotgun quantification across many lipid classes and species is consistent across laboratories and instrument set ups. We argue that at least one major contributing factors is a rationale hardware configuration, where even most basic calibration protocol delivers stable and nearly optimal indices of analytical performance that share striking similarity between instruments and laboratories. For the same reason it is also possible to benefit from extended instrumentation capabilities e.g. ultra-high mass resolution - without compromising analytical reproducibility and quantification capacity. We demonstrate that ultra-high mass resolution supports flexible isotopic labeling strategies to monitor the turnover of major classes and species of glycerophospho- and sphingolipids in cell cultures, but also in living animals across their entire life span.

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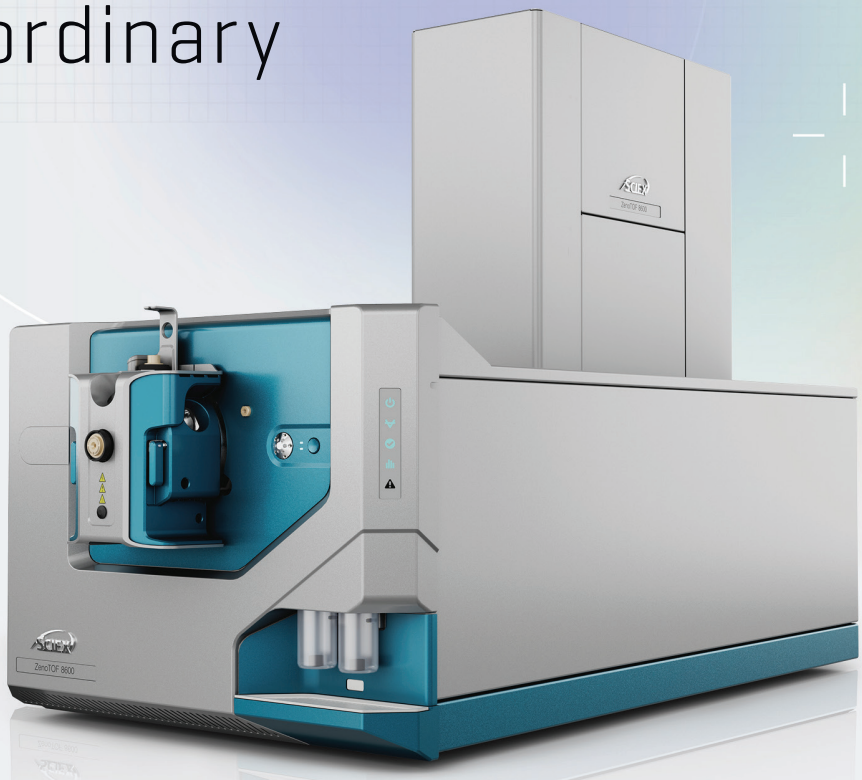

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Abstracts of Posters

P01		Elucidating the Role of Neurotoxic 1-Deoxysphingolipids in GBA1-Parkinson's Disease Mitochondrial Dysfunction	Alecu, Irina
P02		Structural Characterisation of lipids in different disease models using Oxygen Attachment Dissociation (OAD) MS/MS	Armitage, Emily
P03		Application of Shotgun Lipidomics to Rare Diseases	Ali Asgari, Mohsen
P04		LC-MS-based lipidomics with chemometrics feature selection captures plasma lipid shifts after high intensity physical training	Aurum, Fawzan S.
P05	PRIZE	Analysing the phospholipid composition of magnetotactic bacteria through shotgun lipidomics	Badin, Elisa
P06		Determination of fatty acid composition in murine fatty liver samples by GC-HRMS	Bennett, Alexandre
P07		Plasma lipidomics in patients with heart failure undergoing metabolic correction	Berezhnoy, Nikolay V.
P08	PRIZE	Expanding Chemical Space Coverage in Biological Matrices Through Orthogonal Ionization Techniques	Brenner, Christina
P09		Cross-Organ Lipidome Remodeling in Myocardial Ischemia and Reperfusion	Coman, Cristina
P10	PRIZE	The First Cut is the Deepest: In-Depth Glycosphingolipid Characterization in Human Mesenchymal Stem Cells Using 2D-LC-HRMS	Dowlati Beirami, Amirreza
P11	PRIZE	Mechanistic investigation of the influence of unsaturated fatty acids on platelet activation using direct infusion and LC-MS/MS	Eder, Alexander
P12	PRIZE	Altered lipidomic profile in an astrocytoma cell model of Alexander Disease	Fernàndez Bernal, Anna
P13	PRIZE	Deciphering the Lipidome of M. tuberculosis: Culture-Dependent Variations in Virulence Lipids and Membrane Components	Gauda, Wiebke
P14		Quantification of the LPS/phospholipid-ratio in Gram-negative bacteria	Gisch, Nicolas
P15		Brain and Systemic Lipid Dysregulation Associated with Late Onset Alzheimer's Disease: From mouse to man and back again	Griffin, Julian
P16		Automated Multi-Matrix High Throughput Platform for Oxylipin Analysis in Plasma, Bronchoalveolar Lavage Fluid, and Urine	Hagn, Gerhard
P17		Improved sensitivity and reduced false annotations in Lipidomic Applications on Orbitrap Excidion Pro Hybrid Mass Spectrometer	Halikias, Konstantin
P18		Lipid A profiling by MALDI-MS/MS utilizing trapped ion mobility spectrometry	Hayen, Heiko
P19	PRIZE	LC-MS/MS-based characterisation of phosphoethanolamine glucosylceramides and their derivatives in Caenorhabditis elegans	Hillebrand, Julia
P20		Enabling FAIR Comparative Lipidomics with LipidCompass	Hoffmann, Nils
P21	PRIZE	Quantitative lipid profiling in mouse tissue by RP-HPLC-MS and qMALDI-MSI	Hormann, Felix-Levin
P22	PRIZE	Improving the LipidXplorer Ecosystem: Stabilizing lxPostman and Redesigning LipidXplorer for Future Lipidomics Workflows	Hossen, Md Ballal
P23		Uncovering Mitotype-Specific Lipid Signatures in Plant Cells Through Targeted Mitochondrial Isolation	Jeck, Viola
P24		Confident Annotation of Oxidized Lipids in LC-IMS-MSMS by Systematic Extension of Class-Specific Rules	Häßelbarth, Romy
P25		Homologous Series Extension of GPNAE and HexGPNAE in Caenorhabditis elegans Enhances Annotation Confidence in LC-IMS-MS/MS Data	Kessler, Nikolas
P26	PRIZE	Microsampling vs. Chemical Biopsy: A Comparative Study on Tissue Metabolome Extraction	Kim, Helena

P27		Proposal for a Lipidomics Scoring System	Köfeler, Harald
P28		Extending gene ontology (GO) enrichment analysis for Multi-omics	Kopczynski, Dominik
P29		Strategies for untargeted lipid profiling using ion mobility mass spectrometry and mzmine	Korf, Ansgar
P30		Bile acid as a predictor of kidney graft deterioration function	Korytowska-Przybylska, Natalia
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P34		Characterisation of triacylglycerolsin milk at the double bond positional level using oxygen attachment dissociation (OAD)	Moreau, Stéphane
P35	PRIZE	Investigation of triacylglycerols bearing oxylipins	Mosel, Katja
P36		Comprehensive data acquisition workflow on Orbitrap Astral Zoom MS to achieve deep lipidome coverage with high confidence annotations	Mueller, Andre
P37	PRIZE	Analysis of the cell wall glycolipids of Streptococcus canis	Nehrmann, Anna
P38	PRIZE	GIPCs in Austrian White Wine: Integrating CID/EAD LC-MS/MS and Network Analysis to Uncover Glycosphingolipid Dynamics	Nguyenova, Hoang Anh
P39	PRIZE	Improving Lipid Identification and Quantification: Chromatogram Deconvolution for LC-MS/MS Workflows	Niedermaier, Felix
P40	PRIZE	Chiral LC-ESI-MS: Enantiomeric and Regioisomeric Separation of Triacylglycerides in the Liver of Doxorubicin Treated Rats Using Coupled CHIRALPAK IF3 Columns	Nilsson, Jenny Marie
P41		Enhanced Omics Analysis of Small Molecules Using the ZenoTOF 8600 Platform	Ozbalci, Cagakan
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P43	PRIZE	Lipidomics Reveals Benefits of Germinated Linseed Oil: From Enhanced Nutrional Quality and Oxidative Stability to Tissue-Specific Remodeling	Pointner, Tobias
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P45	PRIZE	Quality control considerations for bioactive lipid species in human whole blood samples	Rubenzucker, Stefanie
P46		Tandem mass spectrometric behavior of ammonium adducts of esters and fatty alcohols and its applications in shotgun lipidomics	Schwudke, Dominik
P47		Sex-Specific Lipidomic Signatures Reveal Nonlinear Aging Trajectories in Humans	Sol, Joaquim
P48		Open-Source Tools and FAIR Data Formats for Mass Spectrometry-based Lipidomics	Srikakulam, Sanjay K.
P49	PRIZE	Dynamic Sphingolipid Remodeling During Adipocyte Differentiation	Troppmair, Nina
P50		Approved drugs + membranes = surprises	Yesylevskyy, Semen
P51	PRIZE	A Pseudo-Targeted MS Workflow for Profiling Plasmalogen-Influenced Lipid Networks in C. elegans	Yue, Yicen

Elucidating the Role of Neurotoxic 1-Deoxysphingolipids in GBA1-Parkinson's Disease Mitochondrial Dysfunction

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P01

Sphingolipid metabolism begins with the condensation of serine and palmitoyl-CoA by serine palmitoyltransferase (SPT). Under certain conditions SPT can also use alanine, resulting in the formation of 1-deoxysphingolipids (1-deoxySLs), whose accumulation in mitochondria underlies multiple peripheral neuropathies. Mutations in GBA1, which encodes for glucocerebrosidase and metabolizes glycosphingolipids, represent the strongest genetic risk determinant of Parkinson's Disease (PD) and are predicted to impact on canonical and non-canonical sphingolipid metabolism. Given that many of the enzymes in the sphingolipid metabolic pathway are involved in 1-deoxySL metabolism and that these lipids are neurotoxic, we sought to determine whether these lipids also accumulate in GBA1-PD, and if they functionally impair mitochondria. We isolated mitochondria from post-mortem cingulate and cerebellum of healthy controls, idiopathic PD (iPD), and GBA1-PD patients using a combined Percoll gradient centrifugation and anti-TOM22 magnetic bead extraction. We used unbiased lipidomic approaches employing nanobore high-performance liquid chromatography-electrospray ionization-tandem mass spectrometry (nLC-ESI-MS/MS) to characterize and quantify 1-deoxySL species in brain tissue and in isolated mitochondria. We applied a novel bioinformatic pathway approach to identify how 1-deoxySL metabolism is disrupted by GBA1 mutation. Mitochondrial function was investigated by Western blot quantification of proteins in complexes I-V of the electron transport chain, superoxide dismutase 2 (SOD2), Parkin, and protein S-glutathinoylation (PSSG). We identified a GBA1-PD specific increase in 1-deoxysphingosine levels in cingulate compared to iPD patients. We furthermore determined at which node of the metabolic pathway 1-deoxySL metabolism was differentially affected in patients with GBA1-PD. The disruption in 1-deoxySL metabolism associated with a decrease in SOD2 levels indicating impaired ROS scavenging capacity in the presence of increased 1-deoxysphingolipids, and a compensatory increase in PSSG, presumably to control rising reactive oxygen species. Elevated 1-deoxySL levels further associated with higher Parkin levels linked to mitophagy that may be explained by 1-deoxySL accumulation resulting in mitochondrial hyperfragmentation. Taken together, these data link impairments in 1-deoxySL metabolism to mitochondrial dysfunction in GBA1-PD. Continued exploration of PD-associated lipid change will lead to the characterization of PD at the molecular level and allow for the development of disease-mechanism associated biomarkers and cause-directed treatment focusing on lipid metabolism.

Structural Characterisation of lipids in different disease models using Oxygen Attachment Dissociation (OAD) MS/MS

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P02

Introduction

The structural characterisation of lipids requires the identification of head group, carbon number and the position(s) of carbon-carbon double bonds (C=C). Locating the position of double bonds is vital since minor structural differences between positional isomers can alter a lipid's biochemical function. Oxygen Attachment Dissociation (OAD) is a novel fragmentation technology that enables the localisation of C=C double bonds in lipids. To evaluate its use in the structural characterisation of lipids, OAD has been applied to enhance the identification of lipids found to be significant in two different studies: searching for potential biomarkers of pancreatic ductal adenocarcinoma (PDAC) in human serum and exploring the effects of ethanol toxicity in mouse liver, pancreas and gut.

Methods

Serum and tissue extracts were analysed using an untargeted reversed-phase high resolution MS/MS method to find phenotypic differences between cases and controls for different disease models (PDAC or ethanol toxicity). OAD-MS/MS was combined with CID-MS/MS (OAciD-MS/MS) to reveal the head group, acyl chain length and the number and position of double bonds in lipids found to be significant through untargeted profiling.

Results

Structurally characterised lipids diminished by PDAC included LPC 18:2(n-6,9), LPC 18:3(n-3,6,9), LPC 20:5(n-3,6,9,12,15), LPE 18:1(n-9), LPE 18:2(n-6,9), PC 14:0_18:2(n-6,9), PC 16:0_20:5(n-3,6,9,12,15), PC 18:0_18:2(n-6,9), PC 18:0_20:5(n-3,6,9,12,15), PC 18:1(n-9)_18:2(n-6,9), PC 18:2(n-6,9)/18:2(n-6,9), PC 18:2(n-6,9)_20:4(n-6,9,12,15), PC O-16:1_18:2(n-6,9), SM 18:1(n-14);O2/16:0 and SM 18:2(n-4,14);O2/16:0. Amongst these dysregulated lipids, omega-6 linoleic acid was revealed as a common constituent in several of them.

In the ethanol toxicity study, PC(22:5(n-6,9,12,15,18)/0:0), PC(16:0_22:5(n-6,9,12,15,18)), PC (O-18:1(n-9)/)0:0) were found to be reduced with ethanol toxicity, while PC(20:5(n-3,6,9,12,15)/0:0), PC(14:0_18:2(n-6,9)) and PC(18:2(n-6,9)/18:2(n-6,9)) were increased in different tissues in response to ethanol exposure.

Conclusions

The enhanced level of structural identification provided by OAciD-MS/MS allows the potential to improve our understanding of the biological roles these lipids play in different disease models.

Application of Shotgun Lipidomics to Rare Diseases

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P03

Abstract:
Disruptions in lipid metabolism are a common feature of many rare diseases and can significantly impact overall lipid composition. As such, comprehensive lipid profiling offers a valuable approach for gaining mechanistic insights and supporting diagnostic evaluation. We present a high-throughput shotgun lipidomics workflow optimized for plasma samples from patients groups, i.e. (1) patients with known enzyme defects and (2) patient with undiagnosed conditions or sysndromes without a name. Lipids were extracted and directly infused using the Advion TriVersa NanoMate, followed by analysis on a Thermo Orbitrap IQ-X mass spectrometer. The use of dual polarity acquisition enables broad lipid class coverage with high reproducibility. In cases with established diagnoses, we examined alterations in cholesterol-associated lipid pathways and compared lipid class levels to control samples. For undiagnosed patients, lipidomic profiles were evaluated against known disease patterns and quality control samples to assist clinicians in identifying potential metabolic abnormalities. This untargeted shotgun lipidomics platform demonstrates strong potential for investigating lipid dysregulation in rare diseases and supporting clinical decision-making.

LC-MS-based lipidomics with chemometrics feature selection captures plasma lipid shifts after high intensity physical training

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P04

High-intensity interval training (HIIT) may cause momentary oxidative stress which accumulates oxidised lipids and alters the lipidome profile in human plasma. However, reliable quantitative and analytical approaches to measure these dynamics remains open for improvement. We combined liquid chromatography tandem mass spectrometry (LC-QqQ and LC-Q-TOF) analysis with rigorous chemometrics pipelines to profile this labile lipid species. As well, we evaluate the efficacy of a polyphenol-enriched flaxseed oil (FO) supplement in the subject diets to suppress the production of oxidised phosphatidylcholines (oxPCs) and oxysterols in human plasma.

Nineteen healthy female subjects completed a randomised crossover trial. They consumed either 5 g FO or water (control) pre physical training. And then the plasma samples were collected pre-exercise, immediately post-exercise, and 15 min post-exercise (4 × 8 paired samples). oxPCs and oxysterols were extracted (MTBE method), separated on C18 UHPLC, and detected by MRM (QqQ) or auto-MS/MS (Q-TOF). A QC-LOWESS approach, labelled internal-standard normalisation and in-house R scripts ensured analytical and statistical robustness. Feature reduction used Orthogonal PLS-DA with 10-fold cross-validation with variables of importance in projection (VIP > 1.0, $p < 0.05$) informed biological interpretation.

The results of this study showed that immediately after HIIT, control plasma displayed significant escalation in five oxPCs (e.g., KOOA-PC >130 %, SONPC >190 %) and two non-enzymatically formed oxysterols (7-ketocholesterol >90 %, 7β-hydroxycholesterol >50 %). At the time of 15 min post exercise, OPLS-DA segregated FO plasma from control samples with high accuracy ($Q^2 = 0.920$) from the lipidomics dataset, and $Q^2 = 0.9$ from the oxPCs. Notably, most perturbations normalised within 15 min post-exercise only in the FO consuming subjects, indicating accelerated redox recovery.

Our integrated analytical computational strategy tracks short lived oxidative lipid perturbations triggered by high intensity physical exercise. This finding also justifies the combined application of untargeted and targeted lipidomics with stringent data analysis workflow for nutritional intervention study. It also confirms that polyphenol enriched FO is a practical dietary antioxidant for physical activity.

Analysing the phospholipid composition of magnetotactic bacteria through shotgun lipidomics

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P05 PRIZE

Magnetotactic bacteria are gram-negative aquatic prokaryotes that orient and migrate according to the geomagnetic field. This ability can be ascribed to magnetosomes, i.e. unique intracellular organelles consisting of single crystals of the magnetic mineral magnetite (Fe_3O_4) that are biomineralized within intracellular vesicles of the magnetosome membrane (MM). The MM is composed of a phospholipid bilayer and a set of magnetosome-specific proteins. While the composition and functions of its proteome has been extensively studied, less is known about the phospholipid composition of the MM and its significance in the magnetosome biosynthesis.

In this study, we explore the lipid composition of magnetospirillum gryphiswaldense (MSR-1), comparing the MM with the inner and outer membrane (IM and OM). To this end, we analysed both, the wild-type bacterium (WT) and a mutant strain (ΔmamB) lacking the ability to form the magnetosome membrane. To investigate the lipid profile of magnetotactic bacteria, the different membrane components were isolated, and the lipids were subsequently extracted. Because membrane isolation yielded only small amounts of material, shotgun mass spectrometry (MS), which is ideally suited to identify and quantify lipid species in low amounts, was employed.

Overall, we identified various PE and PG species, including their lyso- forms. In addition, we identified epoxidized versions of the lipids, likely originating due to the high intracellular iron content of the bacterium. Having identified the phospholipid content of the different membrane components via high resolution tandem MS experiments, the lipids were quantified using suitable internal standards. We thus assessed the lipid class distribution in the different membrane components.

Determination of fatty acid composition in murine fatty liver samples by GC-HRMS

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P06

Metabolic Dysfunction-associated Steatohepatitis (MASH) and Alcoholic Steatohepatitis (ASH) are the leading causes globally of liver disease. An increasingly common factor in liver disease is, in part, attributed to the so-called western diet. We have previously reported investigations into the disease-specific pathomechanisms using a murine model, and here present an investigation into the fatty acid content of murine liver samples exhibiting MASH and ASH. Free and esterified fatty acids were separately quantified as their methyl esters (FAME) using gas chromatography coupled to high-resolution mass spectrometry (GC-HRMS). It was found that the fatty acid profile in the MASH model exhibits a significant difference when compared to ASH and healthy controls.

The availability of accurate and precise analytical data is necessary to assist the interpretation of such analyses. Therefore, we investigated the separation of free and esterified fatty acids within the same sample using the selected liquid-liquid extraction to determine its reliability. Here we found that separate quantitation of free and esterified fatty acids requires great care to avoid confounding results.

Plasma lipidomics in patients with heart failure undergoing metabolic correction

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P07

Cardiovascular diseases (CVD) are prevalent in Kazakhstan and all over the world. Patients with CVD often have accompanying metabolic abnormalities, such as obesity, dyslipidaemia, insulin resistance, inflammation and type 2 diabetes (T2D). The genetic profiling of patients with CVD is done using established research methods, such as genomics and transcriptomics. The investigation of the accompanying metabolic disorders for better characterization of disease and treatments, using methods like lipidomics and metabolomics, is being established as a research direction in Kazakhstan. The characterization of diversity of prevalent types of CVD in Kazakhstan based on profiling of genomes and lipidomes would allow development of nationwide strategies for prevention and treatment.

One of the treatments strategies for patients with CVD is a metabolism correction with tirzepatide. Tirzepatide is a dual glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) receptor agonist. Metabolic correction in diabetic patients with tirzepatide was shown to be effective, therefore use of tirzepatide in patients with CVD is being assessed in clinical trials in Kazakhstan.

This project aims to profile plasma lipidomes from clinical trial participants with heart failure and with heart failure and T2D. Namely, plasma will be collected at week 0 before the therapy, at weeks 4, 8, 12, 16, 28, 41 during the therapy, and at week 72 after the end of the therapy. The assayed lipids will represent lipid classes such as triglycerides (TG), diglycerides (DG), cholesterol esters (CE), cholesterol (COH), phosphatidylcholines (PC), alkenyl ether (Plasmalogen) substituent containing PC (PC-P), alkyl ether substituent containing PC (PC-O), lysophosphatidylcholines (LPC), phosphatidylethanolamines (PE), alkenyl ether (Plasmalogen) substituent containing PE (PE-P), alkyl ether substituent containing PE (PE-O), lysophosphatidylethanolamines (LPE), phosphatidylinositols (PI), ceramides (CER), hexosylceramides (HEXCER), sphingomyelins (SM), and acylcarnitines (AC). Lipidomes of patients will be assessed during the course of the therapy in order to identify novel lipid biomarkers of metabolic correction with tirzepatide. Results of this study will open the way for validation studies of lipid biomarkers in larger cohorts and will establish lipidomics as a research method in Kazakhstan.

Expanding Chemical Space Coverage in Biological Matrices Through Orthogonal Ionization Techniques

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P08 **PRIZE**

High-resolution LC-MS has become increasingly powerful, yet a large part of the features detected in untargeted analyses of biological samples remains structurally uncharacterized. Discussions in recent years within the metabolomics and lipidomics communities have focused on the extent of in-source fragmentation occurring during electrospray ionization (ESI) and the contribution of such fragments to the number of unidentified features. [1-3] Additionally, ESI exhibits inherent selectivity toward ionizing more polar compounds, limiting the chemical space observed in complex samples. These limitations in ionization efficiency restrict both the detectable coverage of the metabolome and lipidome, and the generation of high-quality spectra necessary for confident structural annotation.

In this study, we explore the use of SICRIT (Soft Ionization by Chemical Reaction in Transfer), a dielectric barrier discharge ionization source, as an alternative ionization technology complementary to conventional heated ESI. Using a Thermo Scientific Orbitrap IQ-X platform, we compare the performance of ESI and SICRIT for untargeted LC-HRMS analysis of complex biological sample materials, to evaluate ionization behavior across chemically diverse compound classes.

To explore how alternative ionization strategies can expand the scope of untargeted LC-MS, we present a systematic comparison of ESI and SICRIT. This study focuses on their application to human biological matrices, with the goal of mapping both overlapping and divergent regions of chemical space. Preliminary findings from plasma samples suggest that SICRIT enables the detection of distinct molecular features, including less polar compounds that are often missed by conventional ESI. By characterizing the complementarity of these ion sources, this work aims to expand the analytical window for biological discovery, particularly in poorly explored matrices, and to support future advances in environmental metabolomics, natural product research, and systems biology.

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Cross-Organ Lipidome Remodeling in Myocardial Ischemia and Reperfusion

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P09

Myocardial ischemia-reperfusion (I/R) injury leads to profound metabolic disturbances, but the dynamics of lipidomic remodeling in the heart and systemic organs remain incompletely understood. We investigated region- and time-specific lipidomic changes in a murine model after transient occlusion of the left coronary artery, assessing lipid profiles in both infarcted and remote myocardial regions. Parallel lipidomic analyses of the plasma and liver were conducted to evaluate systemic responses. To translate these findings to a clinical setting, the results where compared with lipidomic profiles from patients with ST-elevation myocardial infarction (STEMI) before and after reperfusion therapy.

Lipidomic changes in the heart emerged within 30 minutes of ischemia, marked by the breakdown of triacylglycerols (e.g., TG 52:3) and accumulation of fatty acyls (DHA, EPA, AA), monoacylglycerols (MG 22:6), and diacylglycerols (DG 18:1_18:2). These lipids continued to rise with prolonged ischemia, contributing to cytotoxic stress while re-esterification of the free fatty acids (FFAs) into TGs partially countered this effect. Upon reperfusion, several of these alterations reversed, indicating a dynamic and adaptive lipid response.

Systemic changes in plasma and liver lipidomes revealed alterations in dihydrosphingosines and PUFAs, suggesting coordinated metabolic adaptations beyond the heart. In STEMI patients, we observed similar shifts in key lipid classes, including increases in TGs, ceramides, and PUFA-enriched phospholipids after reperfusion. Notably, while FFAs increased in mice, they declined in patients, accompanied by elevated dihydroceramides, pointing to persistent oxidative stress and potential differences in injury resolution.

Chemical space modeling revealed a correlation between the experimental and clinical lipid profiles, underscoring conserved lipidomic responses across species.

In conclusion, myocardial I/R triggers rapid, region-specific lipid remodeling in the heart and coordinated systemic responses. The identification of shared lipid signatures in both experimental models and STEMI patients highlights the translational value of lipidomics for understanding myocardial injury and identifying potential therapeutic targets.

The First Cut is the Deepest: In-Depth Glycosphingolipid Characterization in Human Mesenchymal Stem Cells Using 2D-LC-HRMS

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P10 PRIZE

Glycosphingolipids (GSLs) play a critical role in cellular recognition and signal transduction, with their core structures evolving during stem cell differentiation. Analyzing both the glycan and lipid moieties of GSLs is challenging due to their extensive structural diversity, low-abundance isomers, and lack of standards. Mass spectrometry (MS) is essential for comprehensive GSL analysis, especially when combined with separation techniques. We recently demonstrated a liquid chromatography–mass spectrometry (LC-MS) approach in human mesenchymal stem cells (MSCs), highlighting its potential for monitoring differentiation (1). However, despite the structural complexity of GSLs, multidimensional separations have yet to be applied to intact GSL analysis.

This study presents an online two-dimensional liquid chromatography (2D-LC) approach utilizing hydrophilic interaction chromatography (HILIC) and reversed-phase (RP) chromatography, coupled with high-resolution mass spectrometry (HRMS) on the Sciex ZenoTOF 7600, to monitor structural changes in intact GSLs during mesenchymal stem cell differentiation. A HILIC column (Waters Acquity UPLC BEH Amide, 1.7 µm, 2.1 mm X 150 mm) enabled class-level separation and monitoring of glycan changes, while an RP C18 column (Agilent InfinityLab Poroshell 120, 2.7 µm, 3.0 mm x 50 mm) provided detailed structural information on the lipid moiety within a 5 min analysis of each cut. Fractionation on the 2D-LC system (Agilent 1290 Infinity II) was performed in peak-based multiple heart cut mode, guided by diode array detector UV spectra and MS data from HILIC. For peak parking sample loops of 40µL was used and an active solvent modulation (ASM) valve was used with a dilution factor of 3 to produce better peak shapes and prevent solvent incompatibilities. In-depth structural characterization relied on 2D retention time information, accurate mass analysis, and fragmentation patterns from collision-induced dissociation (CID) in negative mode. Data processing and annotation were conducted using Skyline, MZmine, and Lipid Data Analyzer (LDA) for MS1-level identification, MS2-level characterization, and in silico fragmentation-based annotation.

We successfully optimized the 2D-LC-HRMS method using GSL standards. Using this approach, we identified of ten glycosphingolipid classes, including acidic species from the Ganglio series and neutral species from the Globo series. From the separation standpoint, the method enabled separation at the molecular lipid species level, distinguishing glycosphingolipids based on carbon chain length, degree of unsaturation, and hydroxylation.

As the next step, this optimized method will be applied to characterize GSL structural changes in human mesenchymal stem cells upon differentiation. We will use the HILIC class information to monitor quantitative changes on the glycan part during differentiation and perform online cuts to transfer changed GSL classes to the RP dimension enabling in-depth structural characterization of GSLs. This proof-of-concept study demonstrates the potential of 2D-LC-MS for in-depth GSL analysis, with huge potential in advancing medical and analytical applications of GSLs.

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Mechanistic investigation of the influence of unsaturated fatty acids on platelet activation using direct infusion and LC-MS/MS

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P11 PRIZE

Cardiac disease is one of the most relevant causes of death in modern society. In particular, due to a rising average age of the population, cardiovascular deaths have been increasing more than expected from the population growth alone, which underlines the necessity of further research into understanding and combating such diseases. [1] One of the possible approaches is focusing on the prevention of cardiac events. Here, recent clinical studies have shown a positive effect of polyunsaturated fatty acids (PUFAs) such as eicosapentaenoic acid (EPA) on plaque formation and occurrence of cardiac events when supplemented in high-risk patients. [2,3]

Even though clinical guidelines have already been updated to recommend the supplementation of EPA in certain cases, the underlying mechanisms behind the effects described above are still not fully understood. First trials have, however, shown that the reduction in adverse events appears to be independent of other clinical parameters such as the total triglyceride level. It is speculated that a shift in the ratio of EPA to arachidonic acid (AA) can lead to the formation of less potent lipid mediators that overall reduces the amount of platelet activation [4]

Hence, there is a strong need to support clinical decision-makers with mechanistic explanations of the interaction of EPA with the platelet lipidome. Previously, methods for the analysis of signaling lipids have been developed. [5] Direct-infusion experiments can reveal shifts in bulk membrane lipids using an untargeted approach. Together they offer a complete picture of the changes in the platelet lipidome when exposed to stimulants and can be used to track the effect of the treatment of platelets with PUFAs.

First exploratory studies of the platelet lipidome have already been published. [6] These methods were optimized and it could be shown that activated platelets can be reliably distinguished from non-activated platelets using DI-MS. Ex vivo trials have been performed to deepen our understanding of platelet activation, looking at the influence of different stimulants. The interaction of EPA with platelets was examined in further ex vivo experiments using isolated platelets in regard to the effect of such treatments on platelet activation. Going beyond EPA, similar PUFAs such as docosahexaenoic acid (DHA) were also investigated to get a more detailed understanding of their respective roles during platelet activation.

Those first results are a key steppingstone to a better understanding of the suggested cardioprotective mechanisms of EPA. Future investigations are planned to further elucidate the mechanisms at work and thus aid in the design of clinical studies and contribute to the search for more effective medications for high-risk patients. Furthermore, it could be of interest to define new biomarkers for cardiac disease if direct correlations between clinical outcome and concentration in platelets arise in future clinical studies.

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Altered lipidomic profile in an astrocytoma cell model of Alexander Disease

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P12 PRIZE

Abstract

Alexander disease (AxD) is a rare and fatal neurodegenerative disorder caused by mutations in the glial fibrillary acidic protein (GFAP), primarily affecting astrocytes. While the cytoskeletal derangement caused by GFAP mutations has been better characterized, little is known about their impact on lipid metabolism and membrane homeostasis.

We have performed a comprehensive mass spectrometry–based lipidomic analysis of an AxD cellular model (U-87 MG astrocytoma cells expressing GFP-tagged GFAP wt or its pathogenic R239C variant). Over 488 lipid species were quantified and analyzed in terms of abundance, structural properties, functional indices, and class-level behavior.

GFAP-R239C expression profoundly altered the astrocytic lipidome, with a global increase in bioenergetic lipids including acylcarnitines, triglycerides, and cholesteryl esters, indicative of mitochondrial β-oxidation dysfunction. These changes were accompanied by a metabolic shift from oxidation to lipid storage. Structural lipid analysis revealed increased chain length, unsaturation, and fluidity in ACs and TGs, and reduced saturation across key lipid classes. Ether-linked lipids—both phospholipids and triglycerides—were selectively enriched, suggesting an adaptive antioxidant response to oxidative stress. Additionally, glycosphingolipid metabolism was distinctly reprogrammed, with reduced mono- and dihexosylceramides and increased trihexosylceramides, a pattern reminiscent of lysosomal lipid storage disorders.

Our findings reveal that GFAP-R239C expression triggers a broad reorganization of lipid metabolism in astrocytoma cells, involving bioenergetic dysfunction, oxidative stress compensation, and sphingolipid remodeling.

Acknowledgements

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Deciphering the Lipidome of M. tuberculosis: Culture-Dependent Variations in Virulence Lipids and Membrane Components

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P13 PRIZE

The infectious disease Tuberculosis (TB), caused by Mycobacterium tuberculosis (Mtb), has afflicted humans for thousands of years and continues to be a major health emergency, causing over a million deaths annually. The fact that approximately one-third of the world's population is infected with Mtb highlights the pathogen's well-adapted interaction with its host. Especially Mtb-specific lipids of the outer mycobacterial membrane (MOM) play a direct role in intracellular survival, host-pathogen interaction and virulence[1,2]. Phthiocerol dimycocerosates (PDIMs) are the essential virulence lipids and known for its role in the pathogenicity of the bacterium[3]. The loss of PDIM in Mtb cultured in vitro is a well-known phenomenon and poses a challenge in research, as PDIM-deficient mutants exhibit reduced virulence and can distort experimental outcomes[4]. To overcome this problem, the supplementation of propionate or vitamin B_{12} during bacterial growth in vitro enhances the production of PDIMs[5].

In this study, we analysed a genetically well-defined Mtb strain under growth conditions with and without propionate as a PDIM enhancing additive to the standard growth media. In this context, it is of particular interest whether the supplementation influences the overall lipidome and/or lead to the formation of additional PDIM species. In this perspective we adapted our shotgun lipidomics workflow to analyse also PDIMs. This included adaptation on the data acquisition strategy using the Q Exactive Plus Hybrid Quadrupole-Orbitrap mass spectrometer (Thermo Scientific) and in-depth analysis of the tandem mass spectrometric fragmentation pathways. Finally, we automated identification routines using LipidXplorer. We additionally determined the lipid profiles of the pathogen including phosphatidyl-myo-inositols (PI), lyso-phosphatidyl-myo-inositols (LPI), cardiolipins (CL), phosphatidylethanolamine (PE), lyso-phosphatidylethanolamine (LPE), phosphatidylglycerol (PG), lyso-phosphatidylglycerol (LPG) and phosphatidyl-myo-inositol mannosides (PIMs), as well as neutral lipids. It is also of interest, whether Mtb grown with propionate has a higher growth rate and therefore an infection advantage.

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Quantification of the LPS/phospholipid-ratio in Gram-negative bacteria

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P14

The outer membrane of Gram-negative bacteria is composed of phospholipids (PLs) in the inner leaflet and lipopolysaccharides (LPS) in the outer leaflet. Recent studies revealed the existence of regulatory controls that ensure a balanced biosynthesis of LPS and PLs, both of which are essential for bacterial viability. To investigate the impact of specific enzymes in maintaining this LPS/PL homeostasis, reliable quantification methodologies are required. To enable this, we have developed a workflow – using rough-type Escherichia coli strains as model systems – that combines shotgun lipidomics for PL and the Purpald assay (detection of unsubstituted terminal vicinal glycol groups of the sugar residues Kdo and heptose; Lee & Tsai, Anal. Biochem. 1999) for LPS quantification. To ensure a reliable quantification of PLs a customized internal standard mix, adapted to the PL-ratio in E. coli strains as determined by ^{31}P NMR, was generated. The proper LPS quantification was proven by applying first the Purpald assay to LPS isolated from the respective E. coli strains by the phenol–chloroform–petroleum ether extraction method (Galanos, Lüderitz & Westphal, Eur. J. Biochem. 1969). In a second step, it was figured out how bacterial pellets have to be processed to enable correct LPS quantification by this assay. In sum, this results in quantitative LPS-/PL-ratios for wildtype strains that enable now future comparative investigations of mutant strains to shed light on membrane homeostasis and biosynthesis regulation.

Brain and Systemic Lipid Dysregulation Associated with Late Onset Alzheimer's Disease: From mouse to man and back again

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P15

Many of the genes associated with late onset Alzheimer's disease (AD) are also associated with lipid examined the roles of ApoE and ABCA7 in dementia using a combination of human cohort studies, mouse models and cell models to define lipid metabolism alterations that increase the risk of developing AD. Using a metabolome wide association study (MWAS) approach and SNPs known to be associated with AD in the AIRWAVE cohort of 2000 police officers in the UK, we identified strong associations metabolism, including ABCA1, ABCA7, Trem2 and ApoE. When this information is taken together that obesity, type 2 diabetes and cardiovascular disease are also associated with cognitive impairment, clearly lipid metabolism has a major role in brain health. In this study we have between ABCA7 and lactosyl-ceramide metabolism, and ApoE and arachidonic acid (ARA) containing lipids which were replicated in the Rotterdam cohort (2000 people). Pursuing the results identified for ABCA7, we demonstrated using Mendelian randomisation that ceramides were on the causative pathway for AD-risk, while in brain tissue of ABCA7 mice, sphingolipid metabolism was altered particularly for sphingomyelins, ceramides, and hexosylceramides. To understand better which cells were most affected we studied microglia, demonstrating that hexosylceramides increase in activated microglia. To examine associations between ApoEe4 and ARA containing phospholipids, we performed untargeted lipidomic profiling of brain, plasma, and liver from humanised APOE knock-in mice overexpressing human islet amyloid polypeptide (hIAPP) which promotes amyloid plaque formation in the brain. In the 6-month APOE4/4 hIAPP mice, docosahexaenoic acid and ARA-containing glycerophospholipids, including plasmalogens, were decreased in the brain. In contrast in the liver, APOE4/4 expression was associated with increased levels of ARA-derived eicosanoids in the liver. Thus, lipidomics of human and mouse models of late onset AD highlight profound changes in lipid metabolism that contribute to disease progression.

Automated Multi-Matrix High Throughput Platform for Oxylipin Analysis in Plasma, Bronchoalveolar Lavage Fluid, and Urine

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P16

Oxylipins are oxygenated products of mono- and polyunsaturated fatty acids that are formed via both enzymatic and non-enzymatic pathways. Most studies to date have focused on eicosanoids and docosanoids, which include well-known lipid mediators involved in numerous (patho-)physiological processes. However, knowledge of oxylipins and their putative biological functions is still sparse due to the limited availability of the respective standards. The optimal matrix for investigating oxylipin function depends upon the disease under study as well as sample availability. While plasma and serum are the most common for human studies, urine and bronchoalveolar lavage fluid (BALF) are commonly used to investigate respiratory diseases (e.g., asthma, COPD). To further investigate the function of oxylipins, we present here a multi-matrix platform for oxylipin quantification. Matrix-specific extraction protocols for plasma, BALF and urine were optimized on an automated 96-well plate-based solid phase extraction system (Extrahera, Biotage). Extracts were analyzed via reversed-phase liquid chromatography mass spectrometry (LC-MS/MS) (Xevo TQ-XS), with column and mobile phase dependent upon the sample matrix and the platform was validated for all 3 sample matrices. The urine method was tailored to analyze 18 urinary eicosanoid metabolites, while the methods for plasma and BALF screen 216 oxylipins including 46 heavy isotope-labeled standards. In particular, 36 docosapentaenoic acid $(DPA)_{\omega-3}$ and $DPA_{\omega-6}$-derived compounds were synthesized. The MS2 fragment spectra were deposited in the LIPID MAPS database and are freely available in the spectral library. Matrix validation was performed by determination of accuracy, precision, matrix effect, recovery and autosampler stability. We observed median interday precision of ~4, 9 and 5% for the high-quality control samples and ~6, 8 and 8% for the low-quality control samples, respectively. The plasma and BALF methods utilized an 11-point calibration curve ranging from 0.001 to 550 ng/mL. For example, PGD_2 exhibited excellent linearity (R^2 = 0.999) across all 11 calibration levels, from 0.029 to 550 ng/mL, with a limit of detection of 0.029 pg on column and a lower limit of quantification of 0.145 pg on column. The plasma method was also applied to NIST SRM 1950 reference plasma, with quantified oxylipin levels comparable to 10-year-old in-house data. An in-house generated R script was developed for data analysis and visualization and packaged into a shiny app, enabling sample type-specific normalization of the sample concentration matrix and generation of data quality control reports. This comprehensive analysis platform will enable oxylipin quantification in multiple matrixes, providing a useful tool for investigating oxylipin function.

Improved sensitivity and reduced false annotations in Lipidomic Applications on Orbitrap Excidion Pro Hybrid Mass Spectrometer

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P17

Discovery lipidomics using mass spectrometry aims to gather comprehensive insights into the lipid diversity of a sample. The lipidome is characterized by high dynamic range because of the wide range of lipid concentrations in a biological sample. This makes the detection of low abundant lipids in a discovery workflow challenging. Many lipids undergo unintentional MS1 fragmentation which can lead to false annotations of these fragments.

Here we evaluate Thermo Scientific™ Orbitrap™ Excidion™ Pro Hybrid mass spectrometer with extended dynamic range, enhanced sensitivity, and minimal in-source fragmentation for lipidomics to mitigate the above challenges. We also show the utility of Thermo Scientific™ Compound Discoverer™ 3.4 with LipidSearch™ software for grouping of unintentional MS1 fragments to avoid false annotations.

Experiments were performed using bovine liver extracts and lipid standards. Lipids were separated on a Reverse Phase C30 column. The modified instrument allows gas phase fractionation and intelligent MS1 multiplexing schemes with optimized injection times for extended dynamic range (EDR) Orbitrap MS1 scans.

Thermo Scientific AcquireX™ strategy, with deep scan workflow, was used for fragmentation of analytes for confident annotation. The instrument was operated using both the normal as well as using the new EDR functionality on the instrument. The data quality, reliability, and robustness of measurements on the instrument were evaluated through selected spiked standards and endogenous lipids, using metrics such as resolution, mass accuracy, signal response and isotopic fidelity. Significantly reduced un-intentional MS1 fragmentation of the compounds was observed. Utilizing the extended dynamic range feature enabled the detection of low abundant lipids and increased annotation by more than 20%.

Thermo Scientific™ Compound Discovere 3.4 with LipidSearch software was used for data processing. The software is able to group unintentional MS1 fragments with its precursor leading to fewer false annotations. The use of LipidSearch leads to confident annotation of lipids.

Novel Aspect
Lipidomics on Excedion Pro Orbitrap MS with Compound Discoverer 3.4 software for confident annotations with reduction in false annotations.

Lipid A profiling by MALDI-MS/MS utilizing trapped ion mobility spectrometry

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P18

Lipopolysaccharides play a crucial role as an endotoxin in various gram-negative bacteria, such as E. coli, and are located in the outer cell membrane. In addition, they are used in vaccines as adjuvant the immune response stimulation. The pathological effect of lipopolysaccharides is primarily due to its lipid anchor, known as lipid A. Lipid A consists of a diglucosamine backbone containing varying polar head groups and fatty acid residues. The composition of lipid A can impact bacterial endotoxicity and may vary between bacterial strains.

Lipid A determination is analytically challenging due to the variety of species. Direct analysis by MALDI-MS or HPLC-MS/MS are commonly used to study the lipid A composition. HPLC-MS/MS allows for a comprehensive analysis of the entire lipid A profile, but is more time-consuming due to the numerous extraction and purification steps required during sample preparation. In contrast, MALDI-MS is less laborious, albeit less sensitive and does not provide structural elucidation of lipid A, despite its potential for bacterial screening. Therefore, we developed a workflow based on microextraction and subsequent MALDI-MS/MS analysis utilizing trapped ion mobility spectrometry (TIMS) and data processing with mzmine software package.[1]

The additional TIMS dimension served for enhanced sensitivity, selectivity, and structural elucidation through mobility-resolved fragmentation via parallel accumulation-serial fragmentation (PASEF) in parallel reaction monitoring (prm)-mode. Furthermore, automated MS/MS acquisition by adapting the spatial ion mobility-scheduled exhaustive fragmentation (SIMSEF) strategy [2] for MALDI spot analysis was utilized within mzmine framework. It also facilitated robust lipid A annotation through a newly developed extension of the rule-based lipid annotation module, allowing for the custom generation of lipid classes, including specific fragmentation rules. In this study, the first lipid A species' collision cross section (CCS) values were generated, which will enhance high-confidence lipid A annotation in future studies.

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LC-MS/MS-based characterisation of phosphoethanolamine glucosylceramides and their derivatives in Caenorhabditis elegans

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P19 **PRIZE**

As a structurally diverse lipid class, sphingolipids are integral components of the eukaryotic plasma membrane with signalling functions. Alterations in sphingolipid metabolism have been linked to several immune and metabolic disorders, diabetes, neurodegenerative diseases, and cancer.[1] Among the subclasses of complex sphingolipids, phosphorylated glycosphingolipids – such as phosphoethanolamine glucosylceramides (PEGCs) – represent a group of understudied lipids present in the model organism Caenorhabditis elegans (C. elegans), which is used in biochemical and genetic research.[2,3] PEGCs are involved in cholesterol mobilisation, which is essential for the growth and larval development of the nematode. Cholesterol deficiency leads to developmental arrest, a phenotype that can be rescued by PEGCs.[3]

The typically low abundance of PEGCs, together with the complexity of the sphingolipidome, pose significant challenges for sphingolipid class characterisation. To address this issue, we developed a two-dimensional high-performance liquid chromatography (2D-HPLC) approach for lipid separation prior to detection. The PEGC sphingolipid subclass is selectively isolated from other lipid classes and matrix components via hydrophilic interaction liquid chromatography (HILIC) in the first dimension. Further intra-class separation of PEGC species can be achieved by a second chromatographic dimension using reversed-phase (RP-)HPLC. PEGC identification is carried out with mass spectrometric detection based on accurate masses and characteristic MS/MS headgroup fragments.

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Enabling FAIR Comparative Lipidomics with LipidCompass

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P20

Lipids are vital to biological systems, with disturbances in lipid homeostasis linked to various diseases. Despite the advancements in lipidomics, the field lacks a central reference point for the occurrence and concentration of lipids. We introduce LipidCompass, a community-driven repository, aligned with the FAIR principles, to simplify discovery, comparison and visualization of lipidomic data from various sources, opening up new perspectives and potentially advancing the field of lipidomics research. The input data format, mzTab-M, allows study data to be mapped to standardized terminology defined by controlled vocabularies such as NCIT and PSI-MS. The embedded lipid nomenclature converter, Goslin, translates lipid names into the latest lipidomics shorthand nomenclature, facilitating cross-linking to public databases like LIPID MAPS, SwissLipids, and ChEBI. Additionally, data is represented according to the hierarchical levels of the shorthand lipid nomenclature, enabling comparisons of studies measured at different structural resolutions at the most common level. The initial studies included as a starting point encompass more than 11,000 distinct lipids across all hierarchical lipid levels, highlighting LipidCompass's potential for large-scale comparative data analysis. Consequently, LipidCompass serves as a comprehensive knowledge base on qualitative and quantitative lipid diversity across different species, tissues, and conditions. As a central component of the Lipidomics Informatics for Life-Science (LIFS) project, LipidCompass will integrate with services like LipidXplorer and LipidSpace. Collaborations with the International Lipidomics Society will further enhance standardization, integrating support for the lipidomics checklist, leveraging a community-guided reviewing and curation model and thereby solidifying LipidCompass as a reference resource in comparative lipidomics.

Quantitative lipid profiling in mouse tissue by RP-HPLC-MS and qMALDI-MSI

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P21 PRIZE

To fully understand the underlying biochemical processes in disease progression, information about the molecular makeup of biological samples is necessary. Mass spectrometry (MS) is a major driver in modern bioanalytical studies, as it provides a sensitive, robust and versatile analysis platform at molecular level.[1] Depending on sample type and research question, different mass analysers can be combined with multiple ionization interfaces. For the in-depth characterization of biological samples regarding analyte structures, analyte composition and analyte amount, high-performance liquid chromatography coupled to MS (HPLC-MS) has been well established. This method is routinely applied due to its robustness, high accuracy, and precision for quantitation. Although detailed information about the molecular makeup of samples can be obtained, little information about the spatial distribution of said analytes in the samples can be made. However, this information might be crucial to decipher the biochemical context. Microscopy methods can reveal these interactions to some extend although lacking the unbiased molecular information often needed. MS imaging (MSI), such as matrix-assisted laser desorption/ionization (MALDI-)MSI bridges this gap, allowing researchers to collect spatially correlated mass spectra down to cellular resolution.[2] For example, MALDI-MSI has been shown to effectively differentiate between healthy and tumorous tissue.[3] The MALDI process itself and therefore the resulting mass spectra, is strongly influenced by the sample, matrix application, measurement conditions and more, making the method more difficult to use in larger comparative studies and even more, pose great challenges to obtain quantitative spatial data of analytes. Multiple studies focussing on the spatial quantitation of exogenous analytes[4,5] as well as recent efforts to quantify multiple lipid classes have been published.[6] Although promising first steps in the lab and comprehensive considerations for the future have been made, quantitative MALDI-MSI is still an ever-evolving research field with a great potential to aid in biomedical research.

Here we demonstrate our combined quantitative HPLC-MS/MS and MALDI-MSI workflow on heart septa, liver, and kidney samples from mice fed with a normal or a high-fat diet. The latter have been treated with L-arginine methyl ester (L-NAME) and nitro-oleic acid or a control vehicle. Fresh frozen samples are cryo-sectioned at 20 µm thickness (Leica CM1520) and either thaw mounted onto glass slides or deposited into Eppendorf tubes. One section is used for MALDI-MSI, the next three adjacent sections for HPLC-MS/MS analysis. First, quantitative lipidomics was performed on a UHPLC (Vanquish Flex, Thermo Fisher Scientific) equipped with an Ascentis® Express C18 column (150 x 2.1 mm: 2.7 µm, 90 Å, Supelco) coupled to a HESI-orbital trapping mass spectrometer (Exploris 240, Thermo Fisher Scientific). For qMALDI-MSI analysis, EquiSplash (Avanti Polar Lipids) was used as stable isotope labelled standards (SILS) and pneumatically sprayed onto the tissue. To ensure a homogenous and reproducible matrix coverage, 2,6-dihydroxyacetophenon (DHAP) applied by sublimation in vacuum was used. Measurements were performed on a Q Exactive HF (Thermo Fisher Scientific) equipped with an AP-SMALDI5AF ion source (TransMIT GmbH) in positive and negative ion mode at a pixel resolution of 15 µm. Mass spectrometric images, data extraction for further analysis and lipid identification was done in LipostarMSI (Molecular Horizon).

This study allows us to compare the two modalities of HPLC-MS and MALDI-MSI for the characterization of biological samples. On the one hand, we can compare the qualitative information, meaning the number of lipid identification we can obtain from both methods. Due to the different nature of the ionization processes as well as the additional dimension of separation in HPLC-MS compared to the ‘shotgun’ approach in MALDI-MSI, distinct differences are expected. For example, we could show that 38 % of detected Phosphatidylcholines (PCs) are present in both measurements, whereas this value drops to only 10 % for Phosphatidylethanolamines (PEs). In total, 19 % of all lipids could be detected in HPLC-MS and MALDI-MSI. Besides exploring the qualitative variation, we were also able to check for the similarity in the quantitative data, that means we can compare trends in relative and absolute abundances of lipid classes and lipid species in both modalities. We observed similar trends during the statistical analysis, as well as for concentrations of significantly altered lipid species such as PC-O 32:0. During the measurements we implemented stringent quality control measures, such as pooled samples and performance tests for RP-LC-MS/MS and checks for homogeneity, as well as daily measurements of the applied quantity of internal standards during the spraying process for MALDI-MSI.

The presented workflow allows us not only to improve the comparison of different MALDI-MSI measurements using a SILS, but also shows the possibility to relatively quantify and confirm the presence and abundance changes of selected biomarkers for diet-dependent lipidome changes. These results will allow us to better interpret MS data from future studies and put them into biological context.

Improving the LipidXplorer Ecosystem: Stabilizing lxPostman and Redesigning LipidXplorer for Future Lipidomics Workflows

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P22 PRIZE

LipidXplorer and lxPostman are part of an open-source software toolkit supporting shotgun lipidomics workflows [1,2]. LipidXplorer identifies and quantifies lipid species in mass spectrometry (MS) data through the flexible, rule-based Molecular Fragmentation Query Language (MFQL). lxPostman is a specialized postprocessing tool for filtering, merging and sorting results from various MS experiments, enabling automated quantitation and quality control. It offers several output formats for next stage analysis and for comparison tools such as LipidSpace [3] and Lipidome Projector [4].

This poster highlights recent bug fixes and updates in lxPostman for improved output consistency and usability. These patches contribute to a more stable lipidomics data pipeline and improve downstream data analysis. We highlight improvements with regard to quality control, data integration and documentation.

In parallel, a major redesign of LipidXplorer is underway. Future versions of the legacy branch will include a better performing data import process and a new parallelized data analysis strategy that can deal with larger study sizes. We will share our concepts on the new software architecture and data processing engine that will be more performant, user-friendly, and set for foreseeable developments in mass spectrometric instrumentation. Enhancements to the MFQL engine and query handling are also planned, to make the software a fit for both new and experienced users. We plan to integrate LC and IMS into our data model while keeping the overall analysis strategy of LipidXplorer intact.

Together, these updates aim to make the LipidXplorer ecosystem more robust and scalable to allow for more efficient and stable lipidomics research. We look forward to the feedback from the community.

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Uncovering Mitotype-Specific Lipid Signatures in Plant Cells Through Targeted Mitochondrial Isolation

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P23

Mitochondria are dynamic organelles essential for numerous cellular processes, and their dysfunction can impair growth and survival across animals, humans, and plants. Traditionally considered homogeneous, recent evidence suggests that mitochondria differ between and within cells, indicating a division of labor among mitochondrial subtypes (mitotypes). However, the molecular basis of this diversity, including that of lipid composition, remains largely unexplored.

To address this, we developed mRACE, a method for cell-type-specific mitochondrial isolation. It employs a chimeric protein (MTF) that enables targeting, visualization, and biotinylation of mitochondria. Directing MTF and a biotin ligase to specific cells allows selective mitochondrial isolation via biotin-streptavidin binding.

We applied mRACE to isolate mitochondria from two distinct leaf cell types in Arabidopsis thaliana: mesophyll cells, specialized for photosynthesis, and guard cells, which regulate gas exchange. Isolated mitochondria were analyzed using non-targeted lipidomics via LC-MS/MS with 4D-TIMS-PASEF acquisition (Bruker Daltonics). Lipidome data were processed using rule-based annotation and statistical evaluation in MetaboScape 2025.

Our lipidomic analysis revealed distinct lipid profiles between mesophyll and guard cell mitochondria, indicating cell-type-specific lipid remodeling. Differential enrichment of lipid classes reflected unique metabolic and respiratory demands of each cell type.

These findings uncover a new layer of mitochondrial specialization and demonstrate the power of combining mRACE with high-resolution lipidomics. This approach reveals new ways of understanding how the composition of lipids contributes to mitochondrial function and cellular identity.

Confident Annotation of Oxidized Lipids in LC-IMS-MSMS by Systematic Extension of Class-Specific Rules

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P24

Lipids can be systematically characterized by their head groups, side chains, double bonds, and modifications, forming structured patterns in LC-IMS-MS/MS data. These structural features give rise to distinct patterns in LC-IMS-MS/MS data, including characteristic MS/MS fragments and neutral losses that facilitate the identification of lipid species. Notably, side-chain-specific fragments and neutral losses enable the determination of individual chain lengths and degrees of unsaturation. Additionally, lipid species exhibit predictable behaviour in both retention time (RT) and collision cross section (CCS), further supporting their annotation.

In this study, we present an enhanced rule-based approach for lipid annotation that extends an existing framework to include oxidized lipid species across multiple classes. By incorporating MS/MS spectral data into the annotation process, our method reduces false positives commonly associated with MS1-only identifications. The approach is particularly effective, as oxidized lipid species also form homologous series in molecular formula (and thus m/z), RT, and CCS—approximately mirroring the trends observed in their unmodified counterparts.

To support confident annotation, we introduce an interactive visualization tool that enables the comparison of homologous series with varying degrees of oxidation. This visual context strengthens the interpretability of lipidomic data and supports the annotation confidence achieved with the extended rule-set described above.

Methods: Oxidized lipid extracts mainly of triacylglycerols (TGs) were analyzed on a timsTOF HT mass spectrometer with PASEF® technology using the 4D-Lipidomics method in positive ionization mode. The system was coupled to liquid chromatography for improved separation. Data processing and annotation was performed using MetaboScape® 2025b software.

Homologous Series Extension of GPNAE and HexGPNAE in Caenorhabditis elegans Enhances Annotation Confidence in LC-IMS-MS/MS Data

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P25

Lipids can be systematically characterized by their head groups, side chains, double bonds, and modifications, forming structured patterns in LC-IMS-MS/MS data. We present a workflow that detects and extrapolates homologous series based on molecular formulas, retention times (RT), and collision cross sections (CCS), revealing yet unannotated lipid candidates and validation of annotated ones. This approach semi-automatically derives and visualizes generic homologous series, here applied in Caenorhabditis elegans lipid extracts, predicting additional potential members with extrapolated RT and CCS values.

C. elegans samples were extracted with H_2O/MeOH (v/v, 50/50) and analyzed using a Waters Acquity UPC system coupled to a Bruker timsMetabo for LC-TIMS-PASEF. Lipid separation was achieved via a BEH C18 column with an H_2O/ACN gradient, and data were acquired in both positive and negative ionization modes. Data processing and extrapolation was performed using MetaboScape 2025b (Bruker). Initial annotations of GPNAEs and HexGPNAEs were based on manually curated target lists, while other lipids were annotated using rule-based methods.

GPNAEs and LPE represent isomeric structures, which require chromatographic separation. Though they show different fragmentation behaviour, specific members show only low abundance for which no or only sparse fragmentation spectra can be obtained. In the separation method used in this study, GPNAEs can be differentiated from LPEs, and both form independent homologous series.

Homologous series were semi-automatically constructed in MetaboScape 2025b (Bruker) by selecting known lipid species, followed by automated identification of repeating units within their molecular formulas. These series were then extended in both directions to predict additional members. RT and CCS values for the extrapolated candidates were estimated, and 95% confidence intervals were calculated and visualized.

Employing this novel workflow, we were able to annotate several novel GPNAE and HexGPNAE species, missed during initial annotation and supported by peers of their respective homologous series. Further validation of the newly added lipid species was performed using MassQL, employing characteristic MS/MS fragments for GPNAE and HexGPNAE.

Microsampling vs. Chemical Biopsy: A Comparative Study on Tissue Metabolome Extraction

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P26 PRIZE

Introduction

Minimally invasive techniques are essential for tissue analysis in medical applications, yet they remain a significant challenge. Microsampling, which relies on very small tissue volumes, has emerged as a promising solution. A typical strategy involves removing a small tissue section, followed by homogenization and liquid-liquid extraction (LLE). Alternatively, chemical biopsy using solid-phase microextraction (SPME) offers a non-destructive method. This approach uses a sorbent-coated fiber or needle inserted directly into the tissue for a set duration to adsorb analytes, which are then desorbed into a solvent for analysis. SPME is particularly advantageous for in vivo analyses.[1] In this study, we investigated and compared the effectiveness of microsampling and chemical biopsy for analyzing selected analytes in muscle tissue based on their analytical performance.

Methods

Pork muscle tissue was selected as the model system, and all samples were prepared in five replicates. For microsampling, 20–30 mg of tissue was collected and homogenized using a bead homogenizer with either methanol or water as the solvent. Isotopically labeled internal standards were added, and LLE was performed following the SIMPLEX protocol.[2] Chemical biopsy was carried out using SPME probes coated with a 1 cm hydrophilic-lipophilic balanced (HLB) sorbent. Sampling was conducted from both intact tissue and tissue homogenates (spiked with isotopically labeled standards), prepared similarly to the microsampling workflow to ensure comparability. After sampling, analytes were desorbed into either acetonitrile/water (1:1, v/v) or isopropanol/methanol (1:1, v/v). Data acquisition employed a dual-HPLC setup using both hydrophilic interaction liquid chromatography and reversed-phase columns, coupled to a high-resolution tribrid mass spectrometer for MS1 and MS2 spectra in both polarities.[3] This comprehensive approach enables the detection of a wide range of compounds, including both metabolites and lipids. Targeted data analysis was performed using Skyline for manual peak integration.

Results

Preliminary results demonstrate that both sampling approaches effectively extracted a broad range of analytes, ranging from polar amino acids to less polar lipids. Microsampling combined with LLE enabled the detection of a greater number of analytes but required tissue consumption and captured both bound and unbound fractions. In contrast, SPME offered non-destructive sampling but was limited to extracting only the unbound analytes. Furthermore, the choice of solvents for homogenate preparation and SPME desorption had a notable impact on analyte intensities and overall sensitivity. Solvent composition was therefore critical to optimizing recovery and signal strength.

Conclusion

Using a dual-HPLC setup coupled with a high-resolution mass spectrometer, both methods enabled comprehensive tissue analysis, each offering a distinct analytical perspective: SPME provides a minimally invasive, non-destructive approach without the need for homogenization, whereas microsampling yields a more exhaustive extraction that includes analytes bound within the tissue matrix.

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Proposal for a Lipidomics Scoring System

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P27

Concomitant with the growth of the lipidomics field in the last decade the necessity for quality standards has also increased. In this respect the official lipidomics shorthand nomenclature reflects the data quality, but does not provide an easy to grasp numerical score. Therefore we propose a lipidomics scoring system which takes into account all the analytical techniques used for lipid identification, such as by mass spectrometry, chromatography, and ion mobility spectrometry and awards scoring points for each of these analytical elements. Importantly, the proposed scoring system is integrated with the official lipidomics shorthand nomenclature by a point score which corresponds to the various annotation levels of the shorthand nomenclature. The advantage of the presented scoring scheme is the abstraction of chemical information into a number, which might be easier to grasp for readers, reviewers and editors alike. The added benefit of such a system is, that it can also be used as an internal quality control system and in this respect might function like a corrective for reconsideration of internal data quality standards.

Extending gene ontology (GO) enrichment analysis for Multi-omics

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P28

Computational multi-omics analysis refers to the integrated analysis of quantitative identification data derived from the three major mass spectrometry (MS)-based omics fields: lipidomics, proteomics, and metabolomics. While recent decades have seen significant progress in the development of tools for analyzing data within individual omics domains [LipidSpace, LipidCreator, Goslin, DAVID, STRING-DB, and various proteomics/metabolomics platforms], truly integrated multi-omics approaches remain in the early stages of development.

To close this gap, we developed the GO multi-omics enrichment analysis platform, the first online tool specifically designed for GO term enrichment analysis across the three major MS-based omics domains: proteomics, lipidomics, and metabolomics. This platform serves as a functional annotation tool, enabling the interpretation of large lists of lipids, proteins, or metabolites by identifying enriched biological features, including Gene Ontology (GO) terms (biological processes, molecular functions, cellular components), metabolic pathways, physicochemical properties, disease phenotypes, and disease associations in one coherent analysis. The platform is a web-based (https://lifs-tools.org/go-lipids) application, designed to simplify the execution of enrichment analyses, open-source, and free of charge.

This novel multi-omics enrichment analysis introduces a powerful new aspect to a comprehensive computational multi-omics analysis pipeline. It establishes direct correlations between study-specific conditions and terms across various domains, such as biological processes, molecular functions, metabolic pathways, and diseases, providing essential insights into the study.

Strategies for untargeted lipid profiling using ion mobility mass spectrometry and mzmine

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P29

High-resolution mass spectrometry, in combination with various coupling techniques such as liquid chromatography (LC) and matrix-assisted laser desorption/ionization (MALDI) of lipidomics samples, generates gigabytes of complex data in a single study. Adding an orthogonal separation dimension through ion mobility spectrometry (IMS) increases overall peak capacity and enables the collection of clean, mobility-resolved MS² spectra, leading to deeper insights per injection. Transforming these highly informative yet complex datasets into knowledge within a reasonable analysis time requires advanced data mining strategies and remains a bottleneck for large-scale studies. Additionally, modern discovery tools such as molecular networking help organize and explore analysis results.

Here, we present novel IMS-enhanced LC- and MALDI-based lipid profiling strategies combined with mzmine[1] data processing and interactive molecular networking. To increase lipid annotation confidence, the workflows include rule-based lipid annotation[2], following the shorthand notation for MS-derived structures[3], and apply multi-dimensional Kendrick mass defect analysis[4]. For the LC-based workflow, we also incorporate equivalent carbon number models to make full use of retention time information. For the MALDI based strategy, we additionally present the spatial ion mobility-scheduled exhaustive fragmentation (SIMSEF) workflow to plan dataset-dependent MS2 acquisition and subsequently acquire MS2 spectra on a timsTOF fleX instrument.[5]

By integrating mzmine with ion mobility-enhanced acquisition strategies, these approaches enable deep, scalable lipid profiling independent of the sampling technology used and within a single software environment.

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Bile acid as a predictor of kidney graft deterioration function

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P30

Background: Kidneys are the most commonly transplanted organ in the world, with the number increasing to 111,135 in 2023 — representing approximately 65% of all organ transplants performed globally. Among kidney recipients, 20-30 experience chronic graft rejection. The gold standard of diagnosing the process of kidney rejection is a biopsy, however it is expensive, invasive and can cause side effects, while markers such as serum creatinine rise only after the injury is present. Metabolomics is a non-invasive tool which could be used to predict deterioration of kidney function.

Aim: The aim of the study was to apply untargeted lipidomics to identify a potential biomarkers for predicting the deterioration of kidney graft function (DoKGF).

Materials and Methods: Plasma of 44 patients of the Nephrology Outpatient Clinic, Department of Immunology, Transplantology and Internal Diseases of the Infant Jesus Clinical Hospital in Warsaw were analysed using LC-MS with Orbitrap Focus. 22 patients have experienced DoKGF, while 22 did not. Plasma was collected within 1-5 years after transplantation, at least 3 months before DoKGF occurred.

Results: By using PLS-DA analysis, one of the bile acid was selected as the compound with the biggest impact on kidney graft function in this group of patients. It was found to be 4 times lower in patients with DoKGF than those without DoKGF ($p=0.002$), with ROC analysis with AUC = 0.724. There was no statistically significant difference in eGFR between groups.

Conclusions: The level of said bile acid may be a potential indicator of DoKGF in kidney recipients. Further studies are required to confirm the accuracy of this compound as a biomarker.

SFC- and HPLC-HRMS based lipid profiling of plants to investigate drought stress responses

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P31 PRIZE

Abiotic stress conditions such as drought, extreme temperatures, and salinity are major factors limiting agricultural productivity worldwide. Due to ongoing climate change, the frequency and intensity of these stressors are expected to increase, posing a growing threat to global resource and food security. In recent years, the role of lipids in plant stress physiology has gained increasing attention. Beyond their functions in energy storage and structural organization of cellular membranes, lipids are integral for signaling processes. Alterations in membrane lipid composition are a central element of the plant's adaptive response, affecting membrane fluidity, permeability, and overall stability under stress conditions. A detailed understanding of lipid dynamics under climate stress is therefore essential for identifying molecular traits associated with stress resilience. This knowledge can inform breeding strategies aimed at developing more robust crop varieties.[1] To facilitate this, we have developed a robust analytical workflow for the in-depth characterization of lipid profiles in plant extracts, enabling insights into stress associated changes in plant lipidomes.

To gain an initial overview of the lipid composition, we applied supercritical fluid chromatography (SFC) hyphenated with high-resolution mass spectrometry (HRMS). This method allowed for efficient lipid class separation and identification, based on both retention behavior relative to reference standards and accurate mass information. The data revealed triacylglycerols (TAGs) as a dominant lipid class in the analyzed plant extracts.

For more detailed insights into TAG remodeling, we subsequently employed reversed-phase high-performance liquid chromatography (RP-HPLC) coupled with electrospray ionization (ESI) and high-resolution tandem mass spectrometry (HR-MS/MS) enabling sensitive and selective detection. Chromatographic separation was based on total number of carbon atoms and double bonds in fatty acid side chains. A comparative analysis of plants grown under drought and control conditions revealed distinct shifts in TAG saturation patterns.

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Application of a fast and reliable cardiolipin focused LC-MS/MS method to murine heart tissue and microglia cell lysates

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P32 PRIZE

Introduction. Cardiolipins (CL) are a phospholipid (PL) group with four fatty acyl substituents, enriched in mitochondria. The multitude of possible fatty acyl chain combinations can lead to significant isobaric overlap, complicating identification and quantitation. Additionally, CL have a 100- to 1000- fold lower abundance compared to other PL classes. Their separation from other PL classes is therefore a requirement for method development. . Large cohort studies to take advantage of the specificity of CL as a biomarker candidate are to date missing from the literature. The analytical challenges posed by CLs have not been overcome in a rapid method enabling high-throughput analysis. In this work, we present a rapid HILIC method capable of robust and reliable CL separation laying the foundation for large scale clinical studies. The method has successfully been applied to two different sample types, murine heart tissue and ultracentrifugation fractions (UCF) of microglia cell lysates. Further, a comparative study between microglia cells cultivated with plant sterols (PS) and normal control (NC) cells was conducted.

Methods. 1 mg aliquots of murine heart tissue C57BL/6 mice were extracted with n-hexane/iso-propanol (IPA) 60/40 v/v. SIM-A9 cells were cultured with PS-enriched medium (12.5 µM campesterol and 12.5 µM sitosterol). After detergent-free lysation and ultracentrifugation, 50 ul of UCFs were prepared by protein precipitation with IPA.

For method development a standard mixture of 7 CL standards (50 ng/ml each) and the SPLASH Lipidomix (diluted 1:180, all: Avanti Polar Lipids, USA) in 97/3 v/v ACN/ aqueous ammonia acetate (AA) buffer (pH 5.8, 15 mM) was used. HILIC separation was achieved on a zwitterionic SeQuant ZIC HILIC (50 x 2.1 mm, 3.5 µm, Merck, DE) with eluents A (97/3 v/v aq. AA buffer (pH 5.8, 15 mM)/ ACN) and B (97/3 v/v ACN/ aq. AA buffer (pH 5.8, 15 mM)). The gradient program was carried out on a Nexera HPLC system (Shimadzu, JP) with a total method runtime of 8.5 minutes. MS/MS acquisition was achieved on a QTRAP 6500 mass spectrometer (SCIEX, CA) in ion switching and multi-reaction monitoring (MRM) mode.

Results. In a first step a rapid separation of 10 PL classes including CL was developed. CL retention on the HILIC was achieved with doubly deprotonated CLs at a pH of 5.8 and minimal water content. CL species had a reproducible mean retention time (tR) of 2.5 ± 0.01 minutes, with the other PL classes eluting between 0.9 minutes (phosphatidylglycerol, PG) and 3.4 minutes (lyso-phosphatidylcholines). Head group separation of the PL classes lead to baseline separation of CL from all tested PL groups except phosphatidic acid, phosphatidylserine (PSer) and phosphatidylinositols (PI).

Intraday repeatability of the MRM transition peak areas for CLs ranged from 1% (CL $(14:1)_4$) to 6% (various CL) and for the other PLs from 1% (PI) to 26% (sphingomyelin, SM).

Application of the method to murine heart tissue revealed the anticipated CL $(18:2)_4$ as the main CL. Comparing heart tissue to the UCF, enrichment of CL in either sample type was species dependent. PS and PI were enriched in the UCF, whereas phosphatidylethanolamines and phosphatidylcholines had comparable intensities between the sample types.

PL classes showed distinctive fraction-dependent patterns in the UCF of cell lysates. Usage of the method in a comparative manner on cells cultivated with PS enriched medium revealed distinctive CL patterns between UCF of control cells and cells treated with PS, with a general enrichment in the NC samples. PSer were enriched in the NC samples, whereas SM were enriched in the PS treated cells. PG and PE had a similar distribution between the groups.

Conclusion. A rapid and robust HILIC separation of PL classes by their polar head group was presented. In the next steps, further sample matrices such as plasma are to be tested, as well as additional validation data is to be measured. Moreover, hyphenation of the HILIC to an orthogonal reversed phase chromatography will lessen the isobaric overlap via CL species separation before mass spectrometric detection. To conclude, the rapidness of the HILIC separation is the key to unlocking large cohort studies of CL in the future.

Lysosomal lipidomics of endothelial progenitor cells in Fabry disease

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P33 PRIZE

Fabry disease (FD) is a lysosomal storage disorder caused by pathogenic variants in the GLA gene, which encodes the lysosomal hydrolase alpha-galactosidase A. This leads to the accumulation of the glycolipid globotriaosylceramide (Gb3) and its deacylated metabolite, globotriaosylsphingosine (lyso-Gb3),within the lysosomes. Given that endothelial dysfunction is a key feature of FD, we used endothelial colony-forming cells (ECFCs) - a subtype of endothelial progenitor cells - as a model for our study.

Our aim was to characterize the lysosomal lipidome in ECFCs of patients diagnosed with FD, to discover low-abundance lipids and lipid-related disease mechanisms beyond the diagnostic lyso-Gb3 marker. Isolating lysosomes from patient-derived ECFCs, rather than analyzing whole-cell lysates - where lysosomes represent a minor fraction - is critical for identifying subtle alterations in lysosomal composition. To our knowledge, this is the first study isolating lysosomes from this cell type, or from patients with this rare disease. Further research into the pathomechanisms of FD is crucial for developing more targeted and effective treatments.

For the isolation of lysosomes, we employed a straightforward yet effective method using superparamagnetic iron oxide particles (SPIONs) that are taken up by cells via endocytosis and accumulate in lysosomes. Lysosomal fractions, whole-cell pellets and the non-magnetic flow-through were subjected to a multi-extraction of lipids, metabolites and proteins and the extracted lipids were analyzed via LC-MS/MS on an Orbitrap Velos Pro. Transition lists were created using LipidCreator software, with additional manually added MS2 chain fragments. Skyline daily was used for lipid identification, and further analysis was performed in R.

The lysosomal lipidomics results of patients diagnosed with FD are pending. Preliminary results from healthy donors revealed over 800 identified lipids in the lysosome, many of which were absent in the non-lysosomal fractions, with notable lysosomal enrichment of lyso-species, such as lysophosphatidylcholine, and plasmalogens. Lysosomal purity was validated by beta-hexosaminidase assays, which showed a consistent ~ 50-fold increase in the lysosomal samples, and by metabolomics data, which showed absence of lactate and presence of cystine in the lysosomal fractions.

Even with preliminary results, our data reveal that isolating lysosomes followed by lysosomal lipidomics uncovers a distinct lysosomal lipidome compared to whole cell fractions, underscoring the value of organelle-targeted analysis. This approach is expected to be powerful for detecting low-abundance lipids that may play key roles in Fabry disease pathology.

Characterisation of triacylglycerolsin milk at the double bond positional level using oxygen attachment dissociation (OAD)

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P34

Introduction

Oxygen Attachment Dissociation (OAD) is a radical-induced dissociation technique which specifically fragments carbon-carbon double bonds (C=C) and can be used to enhance lipid annotation. OAD- and CID-MS/MS can be applied simultaneously in positive or negative ion mode on the LCMS-9050 QTOF (Shimadzu Corporation) to generate specific fragment ions from both. This approach was used to characterise C=C positions in triacylglycerols (TGs)found in a previous profiling study to be differentially expressed in bovine and buffalo milk.

Methods

Milk samples from bovine and buffalo were prepared by the Folch method (200 µL sample + 50 µL water + 1000 µL solvent - chloroform:methanol 2:1 v/v). The bottom, lipid-containing phase was collected, dried, reconstituted in butanol:methanol:chloroform (3:5:4), then diluted 1:50 for analysis. Lipid extracts were analysed by DDA and targeted MS/MS in positive ion mode, with optimal conditions for simultaneous OAD- and CID-MS/MS fragmentation.

Results

OAD-MS/MS specific fragmentation provided evidence of structurally different fatty acids in the milk extracts. For example, the fatty acyl 18:1 was found to be abundant in the n-7 vaccenic acid form as well as the oleic acid n-9 form, which was expected for milk from ruminant species.

As one example, TG 28:1 was predominantly separated into three isomers TG 16:1(n-7)_8:0_4:0 (10.03 min), TG 18:1(n-9)_6:0_4:0 (10.17 min) and TG 18:1(n-7)_6:0_4:0 (10.28 min). The relative proportions of each differed between bovine and buffalo milk extracts. The second two isomers would be indistinguishable using CID alone, highlighting the benefit of OAD in differentiating these lipids based on double bond position (n-9 or n-7).

Conclusion

Given the complexity of lipid distributions in bovine and buffalo milk samples, identifying unresolved TGs is highly challenging. Simultaneous acquisition of CID and OAD specific fragment ions offers a new dimension to characterising and separating isomeric TGs not possible using CID fragmentation alone.

Investigation of triacylglycerols bearing oxylipins

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P35 PRIZE

Polyunsaturated fatty acids (PUFA) are vulnerable to oxidation. This leads to a large number of oxidation products, i.e. oxylipins such as hydro(pero)xy-PUFA and epoxy-PUFA. Endogenously formed oxylipins play an important role in the regulation of inflammatory processes and vascular tone, and are thus involved in the ethology of diseases. Biological effects have mostly been described for free, i.e. non-esterified, oxylipins. However, it has been shown that the majority of oxylipins in biological samples is present esterified, e.g. bound to polar lipids such as phospholipids and neutral lipids such as triacylglycerols (TAG). After intraperitoneal administration of epoxy-PUFA to rats for instance, most of the epoxy-PUFA (60%) were found esterified in neutral lipids, such as TAG, in plasma [(Prostag Leukotr Ess, 2024. **202,** 102622)].

Epoxy-PUFA are potent lipid mediators, which are formed in mammals by cytochrome P450 monooxygenases (CYP). However, recent studies have shown that they are also formed by autoxidation. Currently, the best way to quantify oxylipins in biological samples is by targeted liquid chromatography tandem mass spectrometry (LC-MS/MS). Esterified oxylipins are indirectly determined as free oxylipins after saponification. However, the information about the lipid class in which the esterified oxylipins were bound is lost.

In this project, TAG bearing oxylipins were analyzed by reversed phase liquid chromatography high resolution mass spectrometry (LC-HRMS). For method optimization, standards with the structure TAG 16:0/epoxy-PUFA/16:0 were synthesized. These were used to characterize the fragmentation pattern and retention times of epoxy-TAG. Moreover, edible oils were treated with meta-chloroperoxybenzoic acid (mCPBA) to generate a large variety of potentially biologically occurring epoxy-TAG.

Analyses were carried out using a QExactive HF Orbitrap mass spectrometer operating in Full MS/data dependent MS^2 acquisition mode. Epoxy-TAG were analyzed in positive ionization mode (ESI(+)) and precursor ion were detected as ammonium adducts ($[M+NH_4]^+$). The bound fatty acids and epoxy-PUFA could be characterized by the product ions of the TAG resulting from neutral loss of the (epoxy-)fatty acids and formation of characteristic epoxy-aldehyde fragments.

Comprehensive data acquisition workflow on Orbitrap Astral Zoom MS to achieve deep lipidome coverage with high confidence annotations

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P36

Lipids play a crucial role in cellular processes and disease mechanisms. The use of high-resolution mass spectrometry (HRMS) is essential for lipid identification and characterization. Fragmentation of lipids generate characteristic fragments that help in lipid annotation, structural elucidation, isomer differentiation, and improved confidence in lipid identification. Having fragmentation for most of the lipids detected in a sample hence is highly desirable. This requires fast and sensitive instrumentation. Here we evaluate high resolution Thermo Scientific™ Orbitrap™ Astral™ Zoom mass spectrometer for lipidomics.

Initial experiments were performed using bovine liver extracts and lipid standards. Lipids were separated on a reverse phase C30 column. A mass resolution of 120K was used for the analysis. The data quality, reliability, and robustness of measurements on the instrument were evaluated through selected spiked standards and endogenous lipids, using metrics such as resolution, mass accuracy, signal response and isotopic fidelity. The 30-minute LC-MS method recorded around 250000 MS2 events leading to very high percentage of detected compound being fragmented (i.e., >90%). High quality MS2 fragmentation was observed for very low abundant lipids. The increased sensitivity and fragmentation facilitated the detection of low abundant lipids and increased annotation by more than 10% compared to the Astral Mass Spectrometers and more than 50% compared to traditional mass spectrometers. The optimized parameters were used for data acquisition from 72 mice plasma extracts.

Lipids were extracted from plasma samples obtained from mice fed a high fat diet and mice fed a normal diet. Plasma extracts were analyzed using nanoLC on a Thermo Scientific PepMap™ - column (C18, 75µmx150mm, 2µm) connected to a Thermo Scientific Vanquish™ Neo UHPLC system. The use of nano-flow increased the sensitivity of detected lipids by one to three orders of magnitude depending on the lipid class. It also increased the number of lipid classes detected while achieving a significant reduction in the use of sample, solvent and other resources.

Thermo Scientific™ Compound Discoverer™ 3.4 with LipidSearch™ software was used for data processing. The software is able to group unintentional MS1 fragments with its precursor leading to fewer false annotations. The use of LipidSearch leads to confident annotation of lipids.

Using the workflow, we were able to observe differences in the high fat diet fed mice plasma and the normal diet fed plasma. Preliminary results show that many of the differences were observed in the Triglycerides and Cholesterol Esters.

Novel Aspect
Lipidomics on a modified Orbitrap Astral MS for deeper coverage and confident annotation.

Analysis of the cell wall glycolipids of Streptococcus canis

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P37 PRIZE

Streptococcus canis is an opportunistic pathogen observed in animals, especially dogs and cats, but occasionally also in humans. Usually, this pathogen is classified into the Lancefield group G. Recently, a strain of S. canis belonging to Lancefield group C (IMT52870) was identified. Whole genome sequencing analysis pointed towards a horizontal gene transfer event between S. canis and Streptococcus dysgalactiae in this strain.[1]

Besides the Lancefield assignment, only little is known about cell wall components of S. canis on a molecular level. The aim of this study is to close this knowledge gap by structurally characterizing the lipoteichoic acid (LTA) and glycosylglycerolipids from five selected S. canis strains: four group G strains (G361, IMT49926, AGF1074 and AGF1293) and the group C strain (IMT52870). To this end, we apply our expertise for the isolation and structural characterisation of Gram-positive bacterial cell wall components using mass spectrometry and nuclear magnetic resonance spectroscopy.[2,3]

The obtained results will provide new information on the molecular components of the S. canis cell wall, revealing whether different types of glycolipids are present among different strains and if the exchange of the Lancefield antigen has an impact on the appearance of other cell wall associated molecules.

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[2] Heß, Waldow, Kohler et al., Nat. Communs. **2017**

[3] Gisch, Auger et al., J. Biol. Chem. **2018**

GIPCs in Austrian White Wine: Integrating CID/EAD LC-MS/MS and Network Analysis to Uncover Glycosphingolipid Dynamics

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P38 PRIZE

Glycosyl inositol phospho ceramides (GIPCs) represent a major class of sphingolipids in plants and fungi. Although many biological roles have been established, their specific mechanism and role under environmental stress remains insufficiently understood. In this study, we investigated the temporal response of GIPC in white wine leaf samples (Grüner Veltliner and Riesling) from the Federal College and Research Institute for Viticulture and Pomology Klosterneuburg subjected to artificial heat stress. Following liquid extraction using a solvent system of 2-propanol, n-hexane, and water, and the addition of lactosyl ceramide for relative quantification, high-resolution reversed-phase LC-MS/MS was conducted in CID positive, CID negative, and EAD positive modes. GIPCs were identified with Lipid Data Analyzer (LDA), incorporating a novel implementation of the EAD mode specifically tailored for GIPC analysis. Structural identification was categorized into three confidence levels: (1) MS2 fragment matching across all three acquisition modes, (2) single MS2 confirmation supported by MS1 and retention time, and (3) MS1-based identification with ECN-guided retention time estimation.

So far, we successfully identified 12 GIPC class A lipids with OH functional groups at the highest confidence level, at least three additional A-series lipids with NH and NAc functionalities at the second identification level, and several higher-class GIPC series (D_2HEX_PEN_NAc, D_2HEX_PEN_NH, D_HEX_2PEN_NH, E_2HEX_2PEN_NH and E_3HEX_PEN_NAc) at a tentative level. PCA of the most confidently identified lipids showed clustering depending on the harvesting time, and hierarchical clustering also revealed structural coregulation patterns based on the hydroxylation and unsaturation states. Two-way ANOVA further highlighted differences between wine types, with Riesling showing more dynamic lipid changes over time. A dynamic approach for data analysis was implemented as well to capture time-dependent lipid behavior using the NetworkX Python package. Pearson correlation-based networks were constructed for each time point and condition. Key GIPCs with hydroxylated very-long-chain fatty acids (h-VLFCAs) consistently proved as central hubs, and their biological relevance was determined through eigenvector centrality. Implementing this dynamic view together with static analysis supports the systemic role of h-VLFCAs in membrane stabilization under heat stress.

Improving Lipid Identification and Quantification: Chromatogram Deconvolution for LC-MS/MS Workflows

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P39 PRIZE

Lipidomics workflows typically combine liquid chromatography with tandem mass spectrometry (LC-MS/MS) to identify, and with internal lipid standards to quantify lipids in biological samples. Lipids are first separated with a liquid chromatography (LC) system. Multiple times per second a mass spectrometer measures all precursor mass-to-charge (m/z) ratios. To identify these precursors, consecutively an m/z range is selected and fragmented in the mass spectrometer and the resulting fragments are used to identify and quantify the precursor lipids in this m/z range. Due to lipids consisting of different combinations of the same building blocks, such as polar headgroups and fatty acyl chains, and their predictable fragmentation patterns, different precursors often produce the same fragments. This means, co-eluding precursors with different m/z may create isomer fragments with overlapping chromatograms. Currently in lipidomics, such overlapping fragments are used in an uncontrolled manner and assigned to one of the matching lipids, distorting the identification and quantification.

We developed an algorithm to deconvolute these convoluted fragment chromatograms using the associated precursor chromatograms. With our algorithm we are able to deconvolute strongly convoluted simulated chromatograms accurately and independently of their elution peak shape, and we demonstrate its effectiveness on real experimental data. Accurate deconvolution and attribution of overlapping fragments to their precursors enhances the lipidomics workflow, as more attributed fragments means less misidentifications, and more fragments to compare to the internal lipid standard's fragments means more accurate quantification. Therefore, we can achieve more reliable downstream statistical analyses and biological interpretations.

Chiral LC-ESI-MS: Enantiomeric and Regioisomeric Separation of Triacylglycerides in the Liver of Doxorubicin Treated Rats Using Coupled CHIRALPAK IF3 Columns

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P40 PRIZE

Accurate profiling of triacylglyceride (TG) isomers is essential for understanding lipid metabolism and its dysregulation in disease. While reversed-phase LC-MS (RPLC-MS) offers high sensitivity and detailed fatty acyl profiling, it lacks the ability to resolve regioisomers and enantiomers. These structural variants carry important biological information, particularly regarding stereospecific enzymatic processes. Enzymes such as GPAT-1 initiate TG biosynthesis by catalyzing the stereoselective acylation of glycerol-3-phosphate at the sn-1 position, thereby influencing the enantiomeric ratio of TGs. Thus, enantioselective separation enables a more detailed characterization of lipid biosynthesis and degradation, complementing conventional lipidomics. In this study, we developed a chiral LC-MS method for enantiomeric and regioisomeric separation of TGs in liver homogenates from rats treated with doxorubicin.

Two coupled columns of CHIRALPAK IF3 (250 × 2.1 mm, 3 µm) were used with isocratic elution using acetonitrile/water (98:2, v/v) at 0.15 mL/min at 35 °C using an Acquity-UPLC™ (Waters) system. MS detection was performed using a Synapt G2-S Q-ToF™ (Waters) in positive ESI mode. Post-column infusion of 50 mM ammonium formate enabled the detection of $[TG+NH_4]^+$ adducts. Rats were divided into three groups: control (saline-injected), Dox 6h, and Dox 72h (sacrificed after 6 or 72 h post 10 mg/kg doxorubicin, respectively). Livers were homogenized and extracted using acetonitrile/methanol (10:90, v/v) at −20 °C, followed by reconstitution of the precipitate in isopropanol/acetonitrile prior to analysis.

Method optimization showed that reducing flow-rate and temperature, and adding 2% water to the mobile phase, improved enantiomeric resolution and column efficiency (N). However, due to runtimes of up to 110 min, the temperature was kept at 35 °C to reduce retention time. The optimized method achieved chiral separation in standards of TG enantiomers and regioisomers with varying unsaturation and chain lengths. Fully mono- or di-unsaturated TG enantiomers did not separate well on the column.

Enantiomeric pairs such as sn-OPP (oleoyl-palmitoyl-palmitoyl)/ sn-PPO (palmitoyl-palmitoyl- oleoyl) and sn-LPP (linoleoyl-palmitoyl-palmitoyl)/sn-PPL (palmitoyl-palmitoyl- linoleoyl), revealed shifts in their corresponding enantiomeric ratio. sn-OPP and sn-LPP in the Dox 72h group were both upregulated compared to the control group. These changes suggest stereoselective effects of doxorubicin, particularly when palmitic acid is positioned at sn-2 and sn-3, suggesting an impaired GPAT-1 activity and altered β oxidation.

Greater lipidomic shifts were also observed when studying the TG content of the lipidome. A decrease in α-linolenic acid (18:3) and linoleic acid (18:2) containing TGs, along with increased levels of highly polyunsaturated fatty acids (20:4, 20:5, 22:5, 22:6), was detected in the Dox 72h group, which suggests activation or upregulation of PUFA elongation and desaturation pathways.

This study demonstrates that chiral LC-MS enables detection of stereoselective changes of TG profiles in liver following doxorubicin treatment, alterations not observable by conventional RPLC-MS. The results highlight the importance of resolving TG isomers and enantiomers to better understand alterations in lipid metabolism.

Enhanced Omics Analysis of Small Molecules Using the ZenoTOF 8600 Platform

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P41

Mass spectrometry plays a vital role in lipid and metabolite profiling, with targeted nominal mass workflows traditionally used for quantitation and high-resolution instruments preferred for untargeted discovery. The ZenoTOF 8600 system introduces a new standard by combining high-sensitivity quantitation with advanced structural characterization using both CID and electron-activated dissociation (EAD) fragmentation.

This study presents four examples demonstrating the enhanced performance of the ZenoTOF 8600 in analyzing human-derived lipid and metabolite samples. Compared to the ZenoTOF 7600, the 8600 delivers over a 10-fold increase in sensitivity at both TOF MS and MS/MS levels, significantly improving detection in both targeted (MRM^HR) and untargeted (DDA) workflows.

A key advancement is the ZTScan 2.0 data-independent acquisition (DIA) workflow, which enables broad, high-specificity coverage for small molecule analysis. In DDA mode, the 8600 identified 1.8× more lipids and 1.4× more metabolites than its predecessor. Targeted bile acid analysis demonstrated >10× lower limits of quantitation (LOQs), enhancing analytical sensitivity.

Importantly, the EAD scan mode enabled the generation of unique diagnostic fragments for near-eluting bile acid isomers, allowing for shorter chromatographic gradients and increased throughput without compromising specificity. This capability is particularly valuable for resolving isomeric compounds in complex biological matrices.

Together, these examples highlight the ZenoTOF 8600's ability to unify quantitative and qualitative workflows with exceptional sensitivity, specificity, and structural insight. The integration of EAD and ZTScan 2.0 positions the system as a powerful platform for advancing biomolecular research in clinical, translational, and high-throughput environments.

One injection - Two fragmentation modes: Expanding EAD & CID spectral databases while optimizing fragmentation parameters

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P42 PRIZE

Steroids play an important role in human health and disease[1], however, their analysis by conventional LC-MS/MS workflows remains challenging. Novel fragmentation approaches like electron-activated dissociation (EAD) provide additional layers of information and thus hold great potential for the comprehensive structural elucidation of (sterol) lipids.[2] Yet, spectral databases are sparsely populated with MS/MS spectra originating from EAD fragmentation. We have developed a flow injection-based high-resolution multiple reaction monitoring (MRM^{HR}) workflow that allows for the acquisition of MS/MS spectra at a variety of collision-induced dissociation (CID) and EAD parameters within only four minutes per compound on a latest-generation QTOF mass spectrometer.

The generated spectral library contains high-quality MS/MS spectra for more than 40 steroids, fragmented at ten different collision energies (10-100 V, CID) and nine electron kinetic energies (8-24 eV, EAD) each. EAD and CID MS/MS spectra of steroids differing by only one structural modification were evaluated with ModiFinder[3] to compare the information content of both fragmentation strategies. Applying EAD fragmentation, both the number of fragments that can be explained by in silico fragmentation and the scores for structural modification site elucidation are often higher than for CID-based spectra. A subset of MS/MS spectra was searched against public databases[4] and potentially discovered amongst others in studies on human inflammatory diseases, often treated with the administration of steroid-based drugs. Moreover, we aim to create a list of diagnostic fragments for CID and EAD spectra of steroids, which can be used to search for analogs of interest in previously acquired data sets.

Resulting MS/MS spectra can also be used for parameter optimization, which is inevitable for MRM^{HR} workflows. While conventional parameter optimization requires either multiple LC-MS/MS runs or manual injections (limited to CID fragmentation), the proposed strategy is fully automated and can be used to acquire CID and EAD MS/MS spectra within a single run. These can easily be processed in MZmine for library generation and subsequently be evaluated with an in-house written Python script for MRM^{HR} parameter optimization.

The proposed automated flow injection-high resolution MS/MS pipeline allows for the combination of high-quality MS/MS library generation for CID and EAD fragmentation with MRM^{HR} parameter optimization at high throughput for a variety of compound classes, including steroids.

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Lipidomics Reveals Benefits of Germinated Linseed Oil: From Enhanced Nutrional Quality and Oxidative Stability to Tissue-Specific Remodeling

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P43 PRIZE

The search for sustainable and health-promoting dietary lipids has spotlighted linseed oil from germinated seeds (GLO) as a promising candidate. While its elevated antioxidant capacity and improved oxidative stability have been documented, the metabolic and lipidomic impacts of GLO remain underexplored. We present an integrated study characterizing GLO and examining its influence on lipidomic and metabolite profiles in vivo.

Comprehensive physicochemical and LC-MS analysis of GLO revealed a 37.4-fold increase in total polyphenols and identification of 13 novel phenolic compounds, accompanied by elevated levels of tocopherols, carotenoids, and chlorophylls. Antioxidant capacity was significantly enhanced, supported by improved results in ABTS, DPPH, and FRAP assays. Oxidative stability increased markedly, as evidenced by a 3.8-fold longer shelf-life measured by Rancimat and reduced hexanal/nonanal levels.

To evaluate biological effects, mice were supplemented with conventional or germinated linseed oil as part of a high-fat diet. Untargeted and targeted LC-MS-based metabolomics and lipidomics across liver, white (WAT), and brown adipose tissue (BAT) revealed pronounced tissue-specific alterations. GLO significantly increased acylcarnitines in BAT, suggesting enhanced β-oxidation and thermogenic activity. In WAT, GLO enriched omega-3-rich triacylglycerols and showed lower levels of ceramides, which in excess have been linked to impaired metabolic function, indicating a more favorable lipid profile. Hepatic lipidomics showed elevated polyunsaturated phospholipids—including DHA-containing PE(16:0/22:6)—and reduced potentially harmful intermediates, supporting improved metabolic flexibility. Additionally, targeted LC-MS oxylipin profiling indicated reduced pro-inflammatory mediators and increased anti-inflammatory derivatives, highlighting GLO's role in modulating oxidative stress and inflammation pathways.

Our findings demonstrate that germination enhances the nutritional value of linseed oil. Through lipidomic remodeling and oxylipin modulation, GLO promotes favorable shifts in fatty acid utilization, inflammation, and energy metabolism, underscoring its potential in dietary strategies targeting metabolic health and disease prevention.

Lipid Dynamics and High-Value Lipids During Durian (Durio zibethinus) Ripening and Post-Harvest: An Untargeted Lipidomics Analysis

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P44 **PRIZE**

Durian (Durio zibethinus), the 'king of fruits,' is known for its distinctive flavor, yet research on its lipid composition and metabolism is very limited. This study investigates lipid dynamics in the pulp of the Mon Thong cultivar at five ripening stages using an untargeted lipidomics approach. A total of 343 lipid species from 32 subclasses were detected and identified. The results indicate an increase in triacylglycerol (TG) levels and a decrease in free fatty acids (FA) during ripening. Lysophosphatidic acid (LPA) and lysophosphatidylcholine (LPC) were abundant in the early stages but declined later, whereas phosphatidylethanolamine (PE) showed an increasing trend. Additionally, sulfoquinovosyl diacylglycerol (SQDG) was identified in durian pulp for the first time. The lipid profile of durian pulp is predominantly composed of unsaturated fatty acids, which may contribute to its nutritional value. Furthermore, the presence of phytosterols and triterpenoids highlights durian's potential health benefits. These findings provide valuable insights into the nutritional properties of durian and serve as a crucial resource for future cultivar breeding.

Quality control considerations for bioactive lipid species in human whole blood samples

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P45 **PRIZE**

Lipid species exhibit different stabilities during handling of whole blood samples. Some lipids are prone to metabolization when samples are handled incorrectly, possibly leading to non-reproducible results and possible misinterpretation of clinical lipidomics data[1].

To investigate these effects, we analyzed lipids from 17 different bioactive lipid classes in plasma derived from EDTA whole blood using bioinert reversed-phase liquid chromatography coupled to targeted mass spectrometry[2]. The whole blood samples were exposed to 4 °C or 21 °C between 30 min and 24 h before plasma generation. Especially lysophosphatidic acids and certain sphingolipids exhibited a strong increase already after short incubation periods. However, with increasing incubation time or higher temperatures, other lysophospholipid classes, endocannabinoids and some oxylipin species also showed significant changes in concentration. This underscores the importance of correct and controlled sample handling and transport immediately after blood collection to ensure valid results, especially when analyzing bioactive lipids.

However, lipidomics researchers oftentimes obtain plasma samples where the preceding sample handling and thereby the quality of the plasma samples is unclear. To implement a possible quality control strategy for clinical plasma lipidomics, we therefore offer two QC strategies: In the first strategy, NIST SRM 1950 is used as a reference to infer plasma sample quality. This strategy can be regarded as the ”gold standard” and can easily be implemented by any lab, the only prerequisite is the availability of the NIST SRM 1950. Since this reference material might not be readily available for every lab, we propose a second QC strategy based on the concentration range of a panel of very stable and very labile marker lipids. Providing these two strategies enables every lab to independently asses the quality of obtained plasma samples.

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Tandem mass spectrometric behavior of ammonium adducts of esters and fatty alcohols and its applications in shotgun lipidomics

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P46

Ammonium adducts $[M+NH_4]^+$ are a common observation in the positive ion mode in shotgun lipidomics experiments and gained prominence in the analysis of hydrophobic lipids like triacylglycerols and cholesterylesters (1). Specifically, the analysis of cholesterol via acetylation (2) was the starting point of several methodical developments to incorporate derivatization procedures to attain acetic acid esters of hydrophobic and/or poorly ionizable molecules. We will show some examples how acetylation itself and the formation of ammonium adducts helps to improve mass spectrometric sensitivity of several molecule classes in shotgun lipidomics and discuss the overall tandem mass spectrometric fragmentation.

Recently, advancement of our understanding of the fragmentation of alcohols revealed a new opportunity to structurally characterize long aliphatic chains. In gasphase, a carbocation is formed via a ring intermediate that results in defined fragmentation pattern breaking C-C bonds regularly. This is in principle a new way to identify isolated structural units in long aliphatic chains like methyl groups and double bonds. We will present first examples of fatty alcohols and long chain fatty acid esters and hydrophobic lipids of M. tuberculosis.

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Sex-Specific Lipidomic Signatures Reveal Nonlinear Aging Trajectories in Humans

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P47

Objective
Aging is a non-linear dynamic process characterized by complex molecular changes, including lipidome remodeling. The aim of this study is to characterize changes in the plasma lipidome associated with aging, identify sex-specific lipid signatures, and analyze their impact on the aging process.

Methods
We analyzed 543 lipid species across all major classes using high-resolution mass spectrometry in a well-characterized population of 1,030 adults aged between 50 and 98 years (54% women, 46% men). Additionally, circulating fatty acid composition was assessed via gas chromatography. Linear and nonlinear models were applied to define the lipidomic fingerprints of aging and describe the most significant changes occurring throughout this physiological process.

Results
The results reveal significant age-related changes in ceramide and ether phospholipid metabolism. Moreover, lipidomic alterations allowed us to identify three aging crests (ages 55–60, 65–70, and 75–80) with marked sex differences: the 65–70 peak is predominant in men, while the 75–80 peak is more pronounced in women. Lipid enrichment analysis indicates that acylcarnitines and ether phospholipids are key contributors in defining these peaks, with functional indices suggesting modifications in lipid composition.

Conclusions
This study identifies key lipid signatures associated with aging and highlights notable sex-specific differences. Ether lipids play a central role in aging peaks by supporting metabolic adaptation, whereas sphingolipids are crucial drivers of age-related changes. Our findings suggest a decline in lipid functionality, affecting bioenergetics, antioxidant defense, and cellular identity. These results underscore the importance of sex-differentiated approaches and lay the groundwork for identifying biomarkers and developing strategies for healthy aging.

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Open-Source Tools and FAIR Data Formats for Mass Spectrometry-based Lipidomics

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Mass spectrometry-based lipidomics faces significant challenges in the availability and quality of software, computational workflows and data, especially compared to more established fields like proteomics. To address this, the Lipidomics Informatics for Life Science (LIFS) consortium [1], part of the German Network for Bioinformatics Infrastructure (de.NBI), has developed a suite of open-source tools and FAIR (Findable, Accessible, Interoperable, Reusable) data standards to advance lipidomics research. These tools, available as libraries, standalone and web-based applications, enable processing and analysis of targeted and untargeted lipidomics workflows while promoting collaboration through liberal licensing.

The LIFS tools provide a range of different functions for lipidomics researchers: Goslin [2] is a suite of libraries that convert different lipid nomenclatures to standardized lipid shorthand nomenclature, complemented by a web application with database mappings to LIPID MAPS, SwissLipids, and ChEBI. LipidXplorer [3], a flexible platform for untargeted shotgun lipidomics, uses the MFQL "molecular fragmentation query language" for database-independent lipid identification. lxPostman, an R/Shiny-based tool for post-processing and filtering lipid data, is designed to work with LipidXplorer to enable quantification, noise filtering, MS2 fragmentation analysis, normalization, and nomenclature standardization via Goslin. LipidCreator [4], a user-friendly tool for designing targeted mass spectrometry assays with spectral library generation and collision energy optimization, is available as a Skyline plugin and standalone application. LipidSpace [5] provides functionality for interactive exploration and analysis of lipid datasets using advanced computational methods as a standalone application. Lipidome Projector [6] is a web-based tool for visualizing lipid datasets via neural network-driven vector space embeddings based on structural lipid features. Additionally, the Lipidomics Minimal Reporting Checklist [7] provides a structured wizard for transparent, reproducible data reporting, with PDF export and DOI generation via Zenodo.

By prioritizing FAIR data formats and open-source collaboration, LIFS tools streamline lipidomics workflows, enhance data reproducibility, and foster innovation in life sciences research. The LIFS web portal serves as a centralized and convenient hub for the broader scientific community to discover, access and learn about our tools.

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Dynamic Sphingolipid Remodeling During Adipocyte Differentiation

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P49 **PRIZE**

Adipogenesis is a tightly regulated process by which precursor cells transition into lipid-storing adipocytes. While it is well established that adipogenesis is driven by a transcriptional cascade involving positive feedback loops [1,2], the role of lipid signaling and the contribution of specific small molecules to this process remain incompletely understood.

Sphingolipids (SLs), beyond their role as membrane constituents, are increasingly recognized as bioactive signaling molecules. Using mass spectrometry-based lipidomics, we characterized the sphingolipidome during adipocyte differentiation and observed marked remodeling. Complex glycosphingolipids (GSLs), such as the ganglioside GM3, were downregulated, while lysosphingolipids, particularly sphingosine 1-phosphate, increased substantially.

To probe the functional relevance of GSLs, we inhibited glucosylceramide synthase (GCS), the enzyme generating glucosylceramide from ceramide, using a small molecule inhibitor. This pharmacological perturbation was validated using siRNA, targeting genes encoding GCS as well as two other enzymes involved in SL synthesis. Both interventions led to reduced GSL levels and impaired adipocyte differentiation, as evidenced by lower expression of peroxisome proliferator-activated receptor-γ (PPARG), known as the master adipogenic regulator [3]. Conversely, exogenous GM3 loading via liposomes promoted differentiation, supporting a specific role for gangliosides in adipogenic signaling. In parallel, the rising abundance of lysosphingolipids during differentiation may reflect their function as direct ligands for PPARG, promoting its transcriptional activity. Together, these observations point to a lipid-driven feedback loop coordinating adipogenic commitment.

In summary, we identify dynamic sphingolipid remodeling as a hallmark of adipocyte differentiation, with complex and signaling SL species exerting opposing regulatory effects. By integrating lipidomics with specific perturbation and imaging-based readouts, we highlight how sphingolipid metabolism contributes to the formation of adipocytes from stem cells. These findings open avenues for targeting lipid-mediated regulatory circuits in metabolic diseases such as obesity.

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Approved drugs + membranes = surprises

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P50

Our understanding of how drugs interact with lipid membranes remains superficial despite its critical importance for drug delivery, efficacy and safety. Particularly, the realm of membranotropic drugs - those that spontaneously integrate into lipid bilayers and may alter their physical properties - is largely unexplored. In our recent studies, we successfully identified two previously unknown membranotropic drugs: ticagrelor - a widely used antithrombotic agent, and remdesivir - a novel antiviral that became known as a possible COVID-19 drug. We show that changes in the membrane properties caused by ticagrelor may alter the microenvironment of its own receptor P2Y12 and could modulate its affinity to the ligands, including ticagrelor itself leading to dual action mechanism. Remdesivir is shown to incorporate into the lipid bilayer and nerve terminals influencing excitatory and inhibitory neurotransmission. Obtained results allowed us to hypothesise that unexpected membranotropic behaviour of known drugs could be a widespread phenomenon, but the lack of large-scale dedicated studies makes its prevalence and practical implications unknown.

A Pseudo-Targeted MS Workflow for Profiling Plasmalogen-Influenced Lipid Networks in C. elegans

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P51 PRIZE

Plasmalogens, a subset of ether-linked phospholipids, are well-recognised for their role in maintaining membrane integrity, regulating oxidative stress, and modulating cell signalling pathways. Despite their biological significance, analytical characterisation of plasmalogens in vivo, especially in neurodegenerative models, remains technically challenging due to their vinyl-ether instability, structural diversity, and lack of commercial standards. To address these limitations, we developed a pseudotargeted and structurally resolved mass spectrometry (MS) workflow that combines derivatisation-based chemical enhancement and high-resolution liquid chromatography-mass spectrometry platforms. We constructed a spectral annotation library for key ether-linked species to support broader adoption and standardisation, integrating MS/MS fragmentation patterns of purified standards. This MRM database facilitated the automated identification of plasmalogen subclasses across sample types and experimental conditions, enhancing reproducibility and reducing manual curation efforts.

This study focuses on Caenorhabditis elegans, employing the transgenic Alzheimer's model strain CL4176, which conditionally expresses human Aβ peptide upon thermal induction, and its genetic control CL802. The CL4176 model enables us to investigate lipidome remodelling in a disease-relevant context. Worms were supplemented with purified plasmalogen standards isolated from the marine Ascidian (Halocynthia roretzi), allowing us to evaluate the modulation of ether lipid metabolism and associated stress responses.

Our analytical workflow incorporates two complementary derivatisation strategies. The first involves dimethyl acetal (DMA) derivatisation, enabling the specific conversion of plasmalogen alkenyl chains into identifiable fatty aldehydes, which are subsequently quantified via gas chromatography-mass spectrometry (GC-MS). This provides a rapid and sensitive measurement of total plasmalogen content and fatty chain profiles. The second strategy employs the Paternò–Büchi (PB) photochemical reaction, which introduces structural specificity by labelling double bond positions in alkenyl chains. This reaction is followed by high-resolution quadrupole time-of-flight (HPLC-QTOF) and multiple reaction monitoring (MRM) modes. These dual modes allow us to perform detailed structural elucidation and targeted quantification with high throughput and accuracy.

Results revealed marked differences in lipid remodelling between the two strains. In CL4176, plasmalogen supplementation significantly altered the levels of ether-linked phosphatidylethanolamine (PE) and phosphatidylcholine (PC) subclasses, as well as the unsaturation indices and double-bond localisation patterns of alkenyl chains. These changes were accompanied by increased expression of markers. Specifically, transcriptomic profiling further revealed that plasmalogen treatment downregulates multiple genes associated with inflammation, oxidative stress, and Aβ toxicity while upregulating functional genes involved in immune protection, mitochondrial repair, and neuronal homeostasis. In contrast, CL802 showed relatively modest lipidomic responses, highlighting the disease-state-specific sensitivity to ether lipid supplementation.

The novelty of this study lies in the combined use of dual derivatisation, high-resolution detection, natural ether lipid standards, and genetically defined C. elegans models, enabling a highly resolved view of plasmalogen metabolism under stress conditions. The approach bridges analytical chemistry with functional biology and is relevant to lipidomics research in oxidative stress, ageing, and neurodegenerative disease contexts. In conclusion, our pseudo-targeted lipidomics workflow provides a robust and scalable platform for dissecting ether lipid dynamics in whole-organism models. By linking structural lipidomics with molecular stress phenotypes, this study highlights the functional significance of plasmalogens and offers a blueprint for future investigations into lipid-based modulation of disease pathways.

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