Lipidomics Section
Czech Society for Biochemistry and Molecular Biology

Czech Academy of Sciences

INSTITUTE OF PHYSIOLOGY CAS

9TH CZECH LIPIDOMICS AND METABOLOMICS CONFERENCE

May 18–19, 2026

Conference halls, Czech Academy of Sciences
Národní 3, Prague

PROGRAM

Organizer

Institute of Physiology of the
Czech Academy of Sciences

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PROGRAM

Monday, May 18, 2026

10:30–10:40 **Opening of the Conference**
Tomas Cajka, Ondrej Kuda (*Institute of Physiology of the Czech Academy of Sciences*)

10:40–12:30 **Session 1 – Introduction to Metabolomics and Lipidomics**

10:40–11:40 (60 min) **Tomas Cajka** (*Institute of Physiology of the Czech Academy of Sciences*)
L01 **Tutorial on Metabolomics and Lipidomics: From Sample Preparation to Data Analysis**

11:40–12:30 (50 min) **Oliver Fiehn** (*West Coast Metabolomics Center, University of California, Davis*)
L02 **Have Confidence! Combining Nontargeted and Targeted Metabolomics**

12:30–14:00 **Lunch & Posters**

14:00–15:35 **Session 2 – Analytical Innovations in Metabolomics and Lipidomics**

14:00–14:25 (25 min) **Michal Holčapek** (*University of Pardubice*)
L03 **Bioinert UHPSFC/MS in Comprehensive Lipidomic Quantitation**

14:25–14:45 (20 min) **Ondrej Kuda** (*Institute of Physiology of the Czech Academy of Sciences*)
L04 **Epicardial Adipose Tissue Produces L-3-Hydroxybutyrate in Advanced Heart Failure**

14:45–15:00 (15 min) **Jiri Hricko** (*Institute of Physiology of the Czech Academy of Sciences*)
L05 **Accelerating LC–MS Lipidomics Without Compromising Biology**

15:00–15:25 (25 min) **Simonas Rudys** (*Thermo Fisher Scientific*)
L06 **Integrating Discovery and Targeted Analysis: Combining Orbitrap Astral and Stellar Platforms**

15:25–15:35 (10 min) **David Heywood** (*Waters*)
L07 **High-Throughput Single-Cell Metabolomics Using Standard-Flow LC and a Multi-Reflecting TOF MS**

15:35–16:05 **Coffee Break & Posters**

16:05–17:35 **Session 3 – Metabolomics and Lipidomics in Clinical Diagnostics and Biomarker Discovery**

16:05–16:25 (20 min) **David Friedecký** (*Palacký University and University Hospital Olomouc*)
L08 **Synovial Fluid Lipidomics Enables Accurate Differentiation of Infectious Arthritis Through a Reproducible Host-Response Signature**

16:25–16:45 (20 min) **Jakub Rozhon** (*University Hospital Olomouc*)
L09 **Comprehensive Mass Spectrometry Profiling of Serum in Paediatric Patients with Crohn's Disease and Ulcerative Colitis**

16:45–17:05 (20 min)
Tomas Cajka (*Institute of Physiology of the Czech Academy of Sciences*)
L10 Can We Measure Metformin Adherence? Insights from Plasma Metabolomics

17:05–17:20 (15 min)
Jitka Zrostlíková (*Altium International*)
L11 Bravo Enabled End-to-End LC/MS Metabolomics, Lipidomics, and Proteomics Workflows

17:20–17:35 (15 min)
Alan Barnes (*Shimadzu Corporation*)
L12 Applying Oxygen Attachment Dissociation (OAD) to Identify Positional Isomers of Linoleic Acid Containing Phospholipids Involved in Pancreatic Ductal Adenocarcinoma

17:45–20:00 Dinner

Tuesday, May 19, 2026

09:30–11:00 Session 4 – Lipid Biology, Signaling, and Disease Mechanisms

09:30–09:50 (20 min)
Kristyna Brejchova (*Institute of Physiology of the Czech Academy of Sciences*)
L13 Multi-Tissue Metabolic Reprogramming in Cachexia: The Role of One-Carbon Metabolism

09:50–10:10 (20 min)
Dovilė Milonaitytė (*Institute of Physiology of the Czech Academy of Sciences*)
L14 Searching for FAHFA Transporters in Blood

10:10–10:30 (20 min)
Alena Pecinová (*Institute of Physiology of the Czech Academy of Sciences*)
L15 Polyunsaturated Fatty Acid Sequestration Protects Against Ferroptosis in Mitochondrial Dysfunction

10:30–10:50 (20 min)
Jaroslava Friedecká (*Institute of Experimental Botany of the Czech Academy of Sciences, and Palacký University*)
L16 Time-Dependent Molecular Remodeling of the Vitreous in Retinal Detachment Reveals a Window of Biological Reversibility

10:50–11:00 (10 min)
Tomas Korba (*AMEDIS*)
L17 Fast Quantitative Analysis of Selected Oxylipins with the Resolution of Isomers Using EAD Fragmentation

11:00–11:30 Coffee Break & Posters

11:30–13:00 Session 5 – Cellular Metabolism and Systems Biology

11:30–11:50 (20 min)
Martin Moos (*Institute of Entomology of the Czech Academy of Sciences*)
L18 SAM Transmethylation Pathway and Adenosine Recycling to ATP Are Essential for Systemic Regulation and Immune Response

11:50–12:10 (20 min)
Yuriy Petrenko (*Institute of Experimental Medicine of the Czech Academy of Sciences, and Institute of Physiology of the Czech Academy of Sciences*)
L19 Metabolic Rewiring of Multipotent Mesenchymal Stromal Cells in Three-Dimensional Spheroids

12:10–12:30 (20 min) **Katerina Dadakova** (*Masaryk University*)
L20 Phytochemical Composition of Rowanberries

12:30–12:50 (20 min) **Jan Jurica** (*Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences, and University of Chemistry and Technology*)
L21 Regioselective and Stereoselective Preparation of Ganaxolone Analogues by Turbo-Grignard Conditions

12:50–13:00 (10 min) **Daniel Vláčil** (*Bruker*)
L22 timsMetabo™: Uncompromised 4D-Metabolomics™ and 4D-Lipidomics™ at Depth, Speed and Scale

13:00 Closing of the Conference

13:05–14:00 Coffee Break / Light Lunch

POSTERS (May 18–19, 2026)

Radana Brumarová (*University Hospital Olomouc and Palacký University Olomouc*)
P01 **Plasma Lipidome Signatures Distinguish Early-Onset Hyperuricemia and Gout and Reflect Response to Urate-Lowering Therapy**

Martina Kadláčková (*University Hospital Olomouc and Palacký University Olomouc*)
P02 **Serum Lipid Dysregulation in Systemic Lupus Erythematosus and Lupus Nephritis Indicates Oxidative Stress and Altered Membrane Remodelling**

Vratislav Berka (*Contipro a.s.*)
P03 **Orally Administered Hyaluronan Exerts Metabolic Effects Distinct from Pectin in Healthy Mice**

Antonín Bednář (*University Hospital Olomouc*)
P04 **An Automated Python Workflow for End-to-end Processing and Statistical Analysis of Targeted Metabolomics Data from SciexOS**

Martina Horejšová (*Palacký University and University Hospital Olomouc*)
P05 **Metabolomic Profiling of Honey Bee Hemolymph by LC–MS/MS Reveals Diet-dependent Metabolic Differences**

Jakub Schimmer (*Institute of Physiology of the Czech Academy of Sciences*)
P06 **ViLiFOG: Automating the Construction of Lipidomic Models for Metabolic Flux Analysis**

Marek Wilhelm (Charles University)
P07 **Lipidomic Signatures of Subcutaneous *De Novo* Lipogenesis Track with Ectopic Fat Dynamics during Dietary Carbohydrate Manipulation**

Monika Krakovková (*Institute of Physiology of the Czech Academy of Sciences*)
P08 **Analysis of the Impact of Anesthesia on Metabolic Changes Using a $^{13}C_6$-Glucose Tracer in a Mouse Model**

Tatyana Kobets (*Institute of Physiology of the Czech Academy of Sciences*)
P09 **Relatively High Metabolic Flexibility of Epicardial Adipose Tissue Metabolism in Cardiac Cachexia**

Daniela Nováková (*Charles University and National Institute of Mental Health*)
P10 **Targeted Metabolomic Profiling Reveals Differentiation-Dependent Effects of Methadone in SH-SY5Y Neuroblastoma Cell Line**

Simran Gupta (*Charles University*)
P11 **Integrating Metabolomics and Functional Traits to Uncover Tissue-Specific Responses to Novel Climates in a Grassland Species**

Oleksandr Kozlov (*University of Hradec Králové*)
P12 **Analysis of Triacylglycerol Estolide Stereoisomers by Chiral SFC-MS**

Petr Vodrážka (*Biology Centre CAS*)
P13 **What Do We Really Measure? Impact of Sample Preparation on the Biological Interpretation of Fatty Acid Profiles**

Marie Brezinova (*Institute of Physiology of the Czech Academy of Sciences*)
P14 **Application of mCPBA Derivatization for Structural Analysis of Unsaturated Lipids**

Miroslav Lísa (*University of Hradec Králové*)
P15 **Evaluation of SPE Strategies for Targeted and Untargeted LC-MS Metabolomic Analysis**

Eva Cífková (*University of Hradec Králové*)
P16 **Bioinformatic Approaches for Evaluation of LC-MS Metabolic Changes in Biological Studies**

Eszter Szánti-Pintér (*Institute of Organic Chemistry and Biochemistry of CAS*)
P17 **Identification of Cholesterol Binding Sites on the M_1 Muscarinic Acetylcholine Receptor by Rationally Designed Steroid-dark Quencher Probes**

Vít Kosek (*UCT Prague*)
P18 **Multi-omic Signature of Human Plasma After Acute Coronary Syndrome and Stroke**

Mariia Vodolazhenko (*Institute of Organic Chemistry and Biochemistry of CAS*)
P19 **Activity of Steroids with Atypical Stereochemistry on Ion-channel Receptor**

Tomas Cajka (*Institute of Physiology of the Czech Academy of Sciences*)
P20 **Laboratory of Metabolomics: Services and Analytical Platforms**

9TH CZECH LIPIDOMICS AND METABOLOMICS CONFERENCE

ABSTRACTS

Lectures

L01

Tutorial on Metabolomics and Lipidomics: From Sample Preparation to Data Analysis

Tomas Cajka[1]

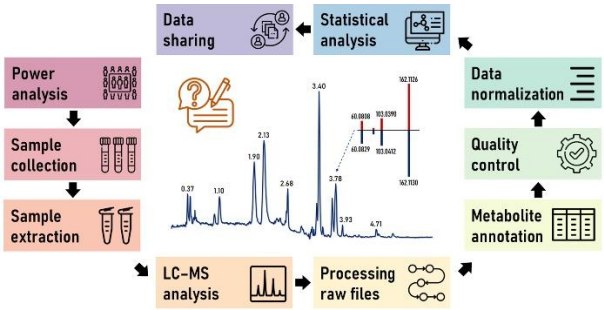
[1] Institute of Physiology of the Czech Academy of Sciences, Prague, Czech Republic

Abstract

The analysis of polar metabolites and complex lipids has become an area of major interest due to growing evidence that dysregulation of these molecules is associated with numerous pathological conditions. Over the last decade, advances in mass spectrometry have significantly transformed metabolomics and lipidomics workflows.

Analytical protocols have been streamlined, and fast-acquisition mass spectrometers now enable the collection of multiple types of mass spectrometric data within a single analytical run. Despite these advances, generating high-quality and reproducible datasets in metabolomics and lipidomics studies remains a major challenge.

In this tutorial, we will discuss key aspects of metabolomics and lipidomics workflows, including current trends and common pitfalls related to: (i) sample type, pre-analytical conditions, and sample preparation; (ii) analytical methods; (iii) data processing and metabolite annotation; and (iv) quality control and data robustness [1–4].



This research was funded by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) funded by the European Union—Next Generation EU.

References

[1] S. Rakusanova, T. Cajka: Tips and tricks for LC–MS-based metabolomics and lipidomics analysis. *TrAC Trends in Analytical Chemistry* **180** (2024) 117940. (doi: 10.1016/j.trac.2024.117940)

[2] S. Rakusanova, O. Fiehn, T. Cajka: Toward building mass spectrometry-based metabolomics and lipidomics atlases for biological and clinical research. *TrAC Trends in Analytical Chemistry* **158** (2023) 116825. (doi: 10.1016/j.trac.2022.116825)

[3] T. Cajka, O. Fiehn: Toward merging untargeted and targeted methods in mass spectrometry-based metabolomics and lipidomics. *Analytical Chemistry* **88** (2016) 524–545. (doi: 10.1021/acs.analchem.5b04491)

[4] T. Cajka, O. Fiehn: Comprehensive analysis of lipids in biological systems by liquid chromatography–mass spectrometry. *TrAC-Trends in Analytical Chemistry* **61** (2014) 192–206. (doi: 10.1016/j.trac.2014.04.017)

L02

Have Confidence! Combining Nontargeted and Targeted Metabolomics

Oliver Fiehn[1]

[1] West Coast Metabolomics Center, University of California, Davis, California, USA

Abstract

Nontargeted metabolomic annotations are currently performed by subjective criteria and an uncoherent selection of criteria and mass spectral libraries. While some companies do not disclose any metadata to their annotations, even academics may state that 'in-house libraries' were used. MS/MS data of most studies that are published in the literature are not available on public. At the same time, it is difficult to compare studies because there are no coherent standards for normalizing data to community quality controls or using the same internal standards.

Mass.Wiki is a public web resource that allows users to compare their MS/MS spectra against all annotations in the UC Davis database environment, for both identified and unknown compounds. It allows users to screen for single spectra, or for all MS/MS spectra of a study. Users who use the UC Davis chromatography methods (BEH C18 for lipidomics and BEH Amide for HILIC separations), including details of solvent gradients and internal standards, can directly standardize their experimental retention times to Mass.Wiki entries. Users can also view, comment on, explore and scrutinize the UC Davis Mass.Wiki entries, opening the door for a transparent and argument-based improvement of metabolomic annotations. Mass.Wiki currently offers public use and commentaries for six databases (assays), all in positive and negative ESI mode: HILIC-MS/MS for Orbitrap and TripleTOF (QTOF) mass spectra, and RPLC-MS/MS for Orbitrap mass spectra (lipidomics). Annotations are based on six parameters: accurate mass of the precursor ions, MS/MS entropy similarity matches, retention time prediction, retention times of positive and negative ESI runs, multiple adducts and biological likelihood.

For quantifications, 43 internal standards for HILIC and 76 internal standards for RPLC lipidomics are used. Sample dilutions are used for assessing matrix effects, and calibration curves give factors to adjust for the number of carbons and double bonds in lipids. We also show how to implement SQUAD for exposome analysis in IQX Orbitraps, using t-SIM and ion trap PRMs for targeting low abundant compounds while simultaneously using the Orbitrap for nontargeted discoveries. Mass.Wiki is the first standardized MS/MS metabolomics database that invites both public use and criticism.

L03

Bioinert UHPSFC/MS in Comprehensive Lipidomic Quantitation

Michal Holčapek[1], Ondřej Peterka[1], Petra Peroutková[1], Robert Jirásko[1], Veronika Šubrtová[1], Zuzana Lásko[1]

[1] University of Pardubice, Faculty of Chemical Technology, Department of Analytical Chemistry

Abstract

Comprehensive lipidomic quantitation is analytically challenging due to the extensive structural diversity of lipids, their wide polarity range, and concentration differences spanning several orders of magnitude. While UHPLC/MS is commonly applied, limitations in lipidome coverage and overall analysis time are present. Ultrahigh-performance supercritical fluid chromatography–mass spectrometry (UHPSFC/MS) represents a high-throughput alternative, but its broader use for highly polar and ionic lipid classes has been hindered by undesirable interactions with metal surfaces, resulting in peak tailing and reduced sensitivity. Here, we introduce a UHPSFC/MS method employing a bioinert column that enables rapid, robust, and comprehensive quantitative lipidomic analysis for nonpolar, polar, and ionic lipid classes.

The separation was carried out using supercritical CO_2 with a methanol-based modifier containing volatile additives, providing class-based separation of 37 lipid classes within 7.5 min, including re-equilibration [1]. The use of the bioinert column effectively minimized interactions of phosphate-containing lipids with metal surfaces, leading to improved peak shapes and enhanced sensitivity. The sample preparation involved a modified MTBE extraction combined with a selective hexane removal to reduce the abundant nonpolar TG and CE classes, while maintaining polar and ionic species. The quantitation was performed using class-specific internal standards and LipidQuant 2.1 software [2]. The method was validated according to bioanalytical guidelines using NIST SRM 1950 human plasma, and the obtained concentrations were in good agreement with interlaboratory consensus values, confirming both accuracy and robustness.

The optimized workflow enabled reliable identification of more than 650 lipid species in human plasma, tissues, erythrocytes, and cell lines. In addition to the class-based UHPSFC/MS approach, a complementary reversed-phase (RP)-UHPSFC/MS method was developed to improve separation at the lipid species level and enhance isomeric resolution of less polar lipid classes, further extending analytical coverage. The bioinert UHPSFC/MS platform demonstrates that comprehensive lipid class separation, including analytically challenging ionic species (e.g., phosphatidic acids, lysophosphatidic acids, ceramide phosphates, sphingoid base phosphates), can be achieved within a single short analysis. This approach provides high-throughput lipidomic quantitation suitable for large-scale studies, as illustrated by lipidomic profiling of human plasma in cancer biomarker research [3]. Multivariate statistical models enabled classification of pancreatic cancer patients and healthy controls with sensitivity and specificity exceeding 95%. This work was supported by ERC AdG No. 101095860.

This work was supported by ERC AdG No. 101095860.

References

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[2] Chocholoušková, M. *et al. Chemometr. Intell. Lab. Syst*. **251** (2024) 105169.

[3] Peterka, O. *et al. Com. Med*. **6** (2026) 127.

L04

Epicardial Adipose Tissue Produces L-3-Hydroxybutyrate in Advanced Heart Failure: Direct Analysis of Fat Metabolic Remodeling

Ondrej Kuda[1]

[1] Metabolism of Bioactive Lipids, Institute of Physiology, CAS, Videnska 1083, Prague, Czech Republic

Abstract

Background: Heart failure (HF) progression involves complex metabolic and multi-organ alterations, but the specific adaptations in adipose tissue are not fully understood.

Aims: We aimed to characterize the metabolic remodeling of epicardial (EAT) and subcutaneous (SAT) adipose tissues in HF with reduced ejection fraction (HFrEF), focusing on lipid metabolism, fatty acid oxidation, and ketogenesis.

Methods: Clinical and metabolomic profiling were performed on metabolically stable controls (n = 34), patients with mild HFrEF (n = 45), and severe HFrEF (n = 129). Metabolomics profiling identified over 800 metabolites in EAT and SAT. Clustering and pathway enrichment analyses defined depot-specific metabolic shifts across HF stages, while gene expression analyses provided mechanistic support.

Results: Advancing HF was associated with declining cardiac function, systemic congestion, and a metabolic shift toward catabolism. Metabolomics revealed depot-specific adaptations: SAT transitioned smoothly to enhanced lipolysis, whereas EAT demonstrated impaired triacylglycerol replenishment and disrupted final turn of β-oxidation spiral. Both depots increased reliance on acylcarnitine degradation and lipolysis; however, EAT was uniquely characterized by late-stage impairment in mitochondrial and peroxisomal fatty acid oxidation, leading to elevation of 3-hydroxybutyrate and hydroxybutyrylcarnitine tissue levels. Ex vivo analyses of EAT explants showed significantly increased fraction of L-3-hydroxybutyrate enantiomer, produced by EAT, compared to D-3-hydroxybutyrate enantiomer originating from the liver.

Conclusions: HF progression drives major, depot-specific metabolic remodeling in adipose tissue. In advanced HF, EAT shows impaired fatty acid oxidation and enhanced local production of L-3-hydroxybutyrate in the vicinity of myocardium, highlighting the close metabolic cooperation in nutrient supply between EAT and the heart muscle through the coronary circulation [1].

This work was supported by Programme EXCELES, ID Project No. LX22NPO5104

References

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L05

Accelerating LC–MS Lipidomics Without Compromising Biology

Jiri Hricko[1], Lucie Rudl Kulhava[1], Michaela Paucova[1], Michaela Novakova[1], Veronika Hola[1], Ondrej Kuda[1], Tomas Cajka[1]

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

Abstract

High-throughput liquid chromatography–mass spectrometry (HT-LC–MS) has emerged as an attractive strategy for large-scale metabolomics and lipidomics studies requiring the analysis of hundreds of samples per day [1]. This presentation explores the development and optimization of rapid LC–MS workflows for the separation of polar metabolites and complex lipids without substantially compromising analytical performance or biological interpretation. Methodological adaptations, including gradient scaling, increased column flow rates, optimized electrospray ionization conditions, and streamlined MS/MS acquisition strategies, were evaluated to improve throughput [2,3].

Using reversed-phase and HILIC-based LC–MS platforms, rapid and high-throughput methods achieved substantial reductions in analysis time while increasing daily injection capacity to over 300 injections per day in selected workflows. The impact of accelerated methods on lipid annotation, isomer separation, and biological interpretation was assessed using plasma samples from chow- and high-fat diet-fed C57BL/6N mice analyzed by Orbitrap-based MS and data-dependent MS/MS acquisition.

Despite reduced chromatographic resolution and lower numbers of annotated lipids in the fastest methods, key biological differences between experimental groups remained detectable. The results demonstrate that rapid LC–MS approaches can preserve essential biological insights while dramatically increasing analytical throughput, making them highly suitable for large cohort studies, clinical screening, and systems biology applications.

This research was funded by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) funded by the European Union—Next Generation EU.

References

[1] S. Rakusanova, T. Cajka: Tips and tricks for LC–MS-based metabolomics and lipidomics analysis. *TrAC Trends in Analytical Chemistry* **180** (2024) 117940. (doi: 10.1016/j.trac.2024.117940)

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L06

Integrating Discovery and Targeted Analysis. Combining Orbitrap Astral and Stellar Platforms

Simonas Rudys[1]

[1] Thermo Fisher Scientific, Austria

Abstract

Metabolomics and lipidomics workflows often require a trade-off between discovery depth and quantitative specificity. Here, we present SQUAD (Simultaneous Quantitation And Discovery), integrating high-resolution targeted and untargeted analysis within a unified strategy. Orbitrap Astral enables deep molecular profiling through high-resolution accurate mass MS1 and rapid, sensitive MS/MS acquisition, supporting broad metabolite and lipid discovery. Complementarily, the Stellar platform provides targeted nominal-mass analysis with MS^3 capability, enabling detailed structural characterization of lipids, including isomer resolution and triglyceride acyl chain determination. Applied to complex biological samples, this combined approach enhances molecular coverage, improves structural annotation of lipids, and maintains robust quantitation. Together, these technologies enable comprehensive and scalable metabolomics and lipidomics workflows.

L07

High-Throughput Single-Cell Metabolomics Using Standard-Flow LC and a Multi-Reflecting TOF MS

David Heywood[1]

[1] Waters, Wilmslow, United Kingdom

Abstract

Single-cell lipidomics and metabolomics are increasingly important for uncovering cell-to-cell biochemical heterogeneity often masked in bulk analyses. However, extremely low analyte abundance in individual cells necessitates highly sensitive and robust analytical workflows. HT29 and CaCO2 gastrointestinal and cancer research cell lines were used to demonstrate a unified LC-MS strategy for high-throughput discovery profiling.

Single cells were isolated using the IsoPick microfluidic platform and deposited directly into LC vials. Lipids were extracted with ice-cold isopropanol containing deuterated internal standards, while polar metabolites were extracted with 0.1% formic acid using a validated method. Extracts were analysed on a U(H)PLC system equipped with a 2.1×100 mm phenyl-hexyl column operated at 0.4 mL/min and 70 °C, coupled to a multi-reflecting TOF mass spectrometer running DIA and DDA modes with enhanced duty cycle (EDC).

Processed datasets (LipoStar for lipids, MARS for metabolites) produced more than 200 lipid identifications and over 150 polar metabolites per single cell (n=5), spanning amino acids, organic acids and nucleotides. High-resolution MS1 and MS2 data, boosted by EDC for ~10× higher MS2 signal, improved confidence in structural elucidation. The phenyl-hexyl chemistry eliminated isopropanol from the mobile phase, reducing background signal and enhancing signal-to-noise.

This streamlined LC-MS configuration enables comprehensive single-cell lipidomics and metabolomics without hardware changes, providing an efficient and scalable platform for multi-OMIC discovery studies.

L08

Synovial Fluid Lipidomics Enables Accurate Differentiation of Infectious Arthritis Through a Reproducible Host-Response Signature

Martina Kadláčková[1], Aleš Kvasnička[1,2], David Friedecký[1], Markéta Trajerová[3], Eva Kriegová[3], Radana Brumarová[1], Jiří Gallo[4]

[1] Department of Clinical Biochemistry, Faculty of Medicine and Dentistry, Palacký University and University Hospital Olomouc

[2] Department of Medical Biochemistry, Oslo University Hospital, Norway

[3] Department of Immunology, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc,

[4] Department of Orthopaedics, Faculty of Medicine and Dentistry, Palacký University Olomouc and University Hospital Olomouc

Abstract

Background: Accurate differentiation between infectious and noninfectious arthritis remains a central diagnostic challenge, as currently used microbiological, cytological, and inflammatory assays show incomplete sensitivity and specificity. Molecular host-response signatures within the synovial compartment may offer additional discriminatory power. Given the central role of lipids in inflammatory signaling and immune-cell biology, synovial fluid (SF) lipidomic profiling constitutes a mechanistically grounded strategy for the identification of infection-associated biomarkers.

Methods: Targeted lipidomics of SF was performed using liquid chromatography coupled to tandem mass spectrometry in two independent experiments (n = 250) comprising samples from patients with osteoarthritis (OA), native joint infection (INF), aseptic total joint replacements (OA/TJR), and periprosthetic joint infections (PPI). In total, 378 lipid species across 20 lipid classes were quantified and evaluated using multivariate and univariate statistics. Lipid alterations were integrated into a composite score derived by LASSO (Least Absolute Shrinkage and Selection Operator) logistic regression.

Results: Compared with OA, INF exhibited a reproducible lipidomic signature characterized by depletion of lysophospholipids and enrichment of ether- and vinyl ether–linked phospholipids as well as unsaturated triacylglycerols. A supervised model based on discriminant analysis achieved an accuracy of 0.93 for INF samples and 0.99 for OA samples in the discovery experiment and maintained similar performance in the blinded validation experiment (1.00 and 0.98, respectively). Arthroplasty-associated samples were classified with an accuracy of 0.75 for both OA/TJR and PPI groups. A nine-lipid composite score derived using LASSO logistic regression was applied in the validation experiment. In the discovery experiment, sensitivity and specificity were 0.90 and 0.96, respectively, at the 95th percentile threshold; in the validation experiment, sensitivity was 1.00 and specificity ranged from 0.93 to 0.98 (positive likelihood ratio up to 41.5). The score increased with synovial neutrophil predominance but retained discriminatory performance across cytological strata, indicating metabolic remodeling beyond leukocyte burden.

Conclusions: SF lipidomics enables accurate differentiation between INF and OA arthritis. Upregulation of ether-linked phospholipids together with depletion of lysophospholipid species represents a reproducible biomarker pattern of infectious inflammation. These findings highlight the potential of lipid profiling as a clinically meaningful tool to support rapid decision-making before culture results are available.

The work was supported by the Ministry of Health of the Czech Republic AZV NU21-06-00370, MH CZ - DRO FNOl, 00098892 and European Regional Development Fund-Project "Interdisciplinary Approaches for the Development and Application of New Materials in Medical Practice - New Omic Technologies" (No. CZ.02.01.01/00/23_021/0009224).

L09

Comprehensive Mass Spectrometry Profiling of Serum in Paediatric Patients with Crohn's Disease and Ulcerative Colitis: Implications for Diagnosis and Disease Monitoring

Jakub Rozhon[1], Eliška Ivanovová[1], Barbora Piskláková[1], Aleš Kvasnička[1], Radana Brumarová[1], Jaroslava Friedecká[3], René Lenobel[3], David Friedecký[1,2], Eva Karásková[2]

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[3] Laboratory of Growth Regulators, Faculty of Science, Palacky University Olomouc, Czech Republic

Abstract

Introduction: Inflammatory bowel diseases (IBD), represented mainly by Crohn's disease (CD) and ulcerative colitis (UC), are chronic immune-mediated disorders with an increasing incidence in childhood. Their clinical course is highly heterogeneous, and current laboratory indicators often do not allow sufficiently accurate discrimination between disease forms or reliable assessment of disease activity and treatment response. Combined assessment of serum lipid, metabolite and protein profiles, therefore, represents a promising approach to characterising biological processes associated with IBD and identifying group-level markers for clinical use.

Aims: This study aimed to assess whether integrated analysis of serum lipidomics, metabolomics and proteomics enables more precise characterisation of paediatric patients with CD and UC, differentiation from healthy controls, and identification of changes relevant to diagnosis and disease monitoring.

Methods: The study included paediatric patients with CD, patients with UC and healthy controls. Serum lipidomic and targeted metabolomic analyses were performed using LC-MS/MS (Sciex QTRAP 6500+), covering more than 700 lipids across over 20 lipid classes and more than 110 metabolites. Proteomic analysis of selected samples was performed using nanoLC coupled to high-resolution tandem mass spectrometry on a timsTOF Pro 2 system (Bruker Daltonics), resulting in the identification of more than 150 proteins. The dataset was processed using univariate and multivariate statistical models, including analyses of relationships between lipidomic, metabolomic and proteomic data.

Results: Multiomic serum profiling showed that patients with IBD had a distinct molecular profile compared with healthy controls, with changes being more pronounced in CD. The lipid profile showed a systematic decrease in phosphatidylcholine plasmalogens and sphingomyelins. Increased docosahexaenoic acid (FA 22:6) levels were also detected in all patients with IBD. Metabolomic analysis indicated perturbations in pathways related to arginine metabolism, vitamin-dependent processes and microbiota-derived metabolites. Proteomic data revealed alterations in proteins associated with the systemic inflammatory response, acute-phase processes, the complement system, coagulation, antioxidant defence and lipid transport. An integrated multi-omic assessment suggested links between disrupted lipid metabolism, metabolic

stress, and activation of immune-inflammatory mechanisms, with differences in extent and intensity between CD and UC.

Conclusion: Comprehensive blood serum profiling, based on simultaneous assessment of lipidomics, metabolomics, and proteomics, provides a more detailed insight into the biological background of CD and UC in the paediatric population. The findings indicate that this approach may support more accurate discrimination between IBD forms, improved assessment of disease activity and, in the future, individualised treatment monitoring. The molecular-level changes identified provide a basis for searching for new serum group-level biomarkers with potential application in routine clinical care.

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L10

Can We Measure Metformin Adherence? Insights from Plasma Metabolomics

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Abstract

Metformin is the most widely prescribed antidiabetic drug, yet objective assessment of patient adherence remains challenging. Using untargeted metabolomics and lipidomics, we analyzed plasma from 637 patients with type 2 diabetes (T2D) with confirmed metformin use and 143 nondiabetic controls, annotating 614 metabolites. Patients were stratified by plasma metformin concentration into sub-therapeutic, therapeutic, and supra-therapeutic groups, and associations were evaluated using multiple linear regression and composite metabolite ranking.

Five previously unannotated features were structurally identified as N-lactoyl-amino acids, whose levels correlated strongly with plasma metformin ($\rho = 0.42–0.55$, $p < 0.0001$) and increased up to 7.2-fold in the supra-therapeutic group. Although detected at nanomolar concentrations, these metabolites showed robust, dose-dependent associations with metformin.

Broader metabolic alterations in T2D included elevated lactate, organic acids, and branched-chain amino acids, alongside reduced urea cycle metabolites. Lipidomic profiling revealed increases in saturated triacylglycerols and diacylglycerols, with decreases in cholesteryl esters, sphingomyelins, and phospholipids.

Together, these findings establish N-lactoyl-amino acids as sensitive, dose-responsive plasma biomarkers of metformin exposure. Despite being orders of magnitude less abundant than lactate and their amino acid precursors, they reflect mitochondrial lactate overflow and pharmacodynamic adaptation, providing a promising objective measure of treatment adherence [1].

This research was funded by the Czech Health Research Council (NU20-01-00186), the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) funded by the European Union—Next Generation EU, and the Czech Science Foundation (21-00477S; LC-HDX-MS workflow).

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L11

Bravo Enabled End-to-End LC/MS Metabolomics, Lipidomics, and Proteomics Workflows

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Abstract

This presentation will demonstrate a comprehensive automated method for fractionating polar metabolites, lipids, and proteins from a single 20 µL plasma sample. The method offers flexible configurations, enabling selective collection of metabolites alone or in combination with lipid and protein fractions. Fractionation is performed using the Agilent Bravo Metabolomics Sample Prep Platform with a Captiva EMR–Lipid plate, while protein fractions are further processed using standard methods on the AssayMAP Bravo platform. Quantitative data are obtained by comprehensive targeted lipidomics and metabolomics methods using Agilent 6495 LC/MS. Same instrument can be used for targeted proteomics approaches.

L12

Applying Oxygen Attachment Dissociation (OAD) to Identify Positional Isomers of Linoleic Acid Containing Phospholipids Involved in Pancreatic Ductal Adenocarcinoma

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Abstract

Pancreatic ductal adenocarcinoma (PDAC) is one of the most aggressive cancers with one of the lowest survival rates due to late diagnosis and high resistance to treatment. In previous work potential biomarkers of PDAC were identified in human serum which included a panel of putatively identified linoleic acid containing phospholipids. Since the structural architecture of lipids is directly related to biochemical function, it is essential to characterize and distinguish different isomers to better understand their biological roles. In this current work, the novel radical induced MS/MS technique of Oxygen Attachment Dissociation (OAD) was applied to identify C=C position assignments in these lipids.

Serum samples were extracted and analyzed using a high-resolution reversed-phase LC-MS/MS Q-TOF system. Data-Independent Acquisition (DIA) was used with Collision Induced Dissociation (CID) in an untargeted MS/MS analysis of serum samples. Following statistical analysis, compounds of significance containing C=C bonds were re-analyzed by Data Dependent Acquisition (DDA) combining CID and OAD (OAciD) to determine the C=C positional bonds in each lipid.

The high-resolution metabolomics analysis by LC-CID-MS/MS, identified patient PDAC serum samples to exhibit significantly different lipid profiles compared to healthy serum controls. These were putatively identified as PC(18:1_18:2), PC(18:2_18:2), PC(18:2_20:4), LPC(18:2) and LPE(18:2). For each identification, OAciD was then applied to find the double bond position of each fatty acid moiety using MS-DIAL. OAciD-MS/MS spectra revealed that the potential lipid biomarkers of PDAC found through untargeted metabolomics analysis specifically contained omega-6 linoleic acid (18:2(n-6,9)). This work has shown that the enhanced level of structural identification provided by OAD-MS/MS has the potential to improve our understanding of the biological roles these lipids.

The research project was approved by the Pancreatic Cancer Research Fund Tissue Bank (PCRFTB) Access Committee.

L13

Multi-Tissue Metabolic Reprogramming in Cachexia: The Role of One-Carbon Metabolism

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Abstract

Cachexia is a debilitating wasting syndrome that drives high rates of morbidity and mortality among cancer patients. Although tumor–host interactions and maladaptive metabolic reprogramming are known to play major roles, their mechanisms remain incompletely understood. Here we present a detailed picture of spatio-temporal metabolic alterations that occur during cachexia, combining untargeted metabolomics with ^{13}C-glucose tracing across a range of organs and tumors from male tumor-bearing mice at early, intermediate, and late disease stages. We identified one-carbon metabolism as a tissue-overarching pathway characteristic for metabolic wasting in mice, which is linked to inflammation, glucose hypermetabolism, and muscle atrophy. Notably, the same rewiring of one-carbon metabolism was recapitulated in several additional mouse models of cachexia. Altogether, these findings establish a molecular framework for the coordinated, multi-tissue metabolic response that underlies cancer-induced cachexia, highlighting one-carbon metabolism as a central, tissue-overarching driver of the wasting phenotype.

The work was supported by the Czech Ministry of Education, Youth and Sports (LUAUS24040).

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L14

Searching for FAHFA Transporters in Blood

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Abstract

Fatty acid esters of hydroxyl fatty acids (FAHFAs) are a class of bioactive lipids with anti-diabetic and anti-inflammatory effects [1]. They elicit their effects in many sites within the organism, such as adipocytes, macrophages, intestine and pancreatic islets [2]. FAHFAs can be obtained through diet or synthesised in adipose tissue, kidney and liver [1]. However, it is unclear how synthesised or dietary FAHFAs are transported through the circulatory system. We have investigated candidate vehicles capable of carrying hydrophobic lipids in blood to see if they can transport FAHFAs. Microscale thermophoresis was used to characterise the interaction between serum albumin protein and 9-PAHSA – the dissociation constant of the interaction was too high to be physiologically relevant. We turned to serum particles as the next transporter candidate. Serum lipoproteins and exosomes were each purified using different density ultracentrifugation-based techniques and their protein and lipid content was characterised using proteomics and lipidomics. Proteomics confirmed the identity of the lipoprotein particles. Lipidomics further enforced the characteristic identities of the lipoprotein particles and exosomes, and revealed that chylomicrons and very low-density lipoprotein particles contain FAHFAs, as well as a FAHFA storage molecules triacylglycerol estolides (TG-EST). We established that FAHFAs can be liberated from TG-EST by lipoprotein lipase, an enzyme which normally hydrolizes triacylglycerol present in chylomicrons and very low-density lipoproteins, indicating that FAHFAs and TG-EST are a part of classic lipoprotein transport and delivery function. The data suggests that chylomicrons and very low-density lipoprotein particles are FAHFA and triacylglycerol estolide transporters and distributors in blood serum.

This work was supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104)—Funded by the European Union–Next Generation EU.

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L15

Polyunsaturated Fatty Acid Sequestration Protects Against Ferroptosis in Mitochondrial Dysfunction

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Abstract

Impaired energy production is a hallmark of mitochondrial oxidative phosphorylation (OXPHOS) defects. However, secondary metabolic disturbances also represent an important trigger for pathologies originating from OXPHOS aberrations. We identified that cells with OXPHOS deficiencies accumulate triacylglycerols enriched in polyunsaturated fatty acids (PUFAs), which are stored in lipid droplets. Sequestration of PUFAs is a critical component of a broader stress response, which also includes downregulation of cellular desaturases and upregulation of glutathione peroxidase 4 (GPX4). Here, we demonstrate that this mechanism represents a physiologically relevant protective strategy, manifesting in cells under hypoxic conditions and in fibroblasts derived from patients with primary mitochondrial complex IV deficiency. As a proof-of-principle, we observed elevated levels of PUFA-enriched triacylglycerols in the plasma of patients with Myoclonic Epilepsy with Ragged Red Fibres (MERRF). Our findings reveal a novel protective mechanism against ferroptosis, which preserves membrane integrity when mitochondrial respiration is compromised.

This work was supported by the Czech Science Foundation (GACR 21-18993S), the National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) – Funded by the European Union-Next Generation EU, the Czech Health Research Council (NU22-01-00499) and the Grant Agency of Charles University (GA UK 283423/2023).

L16

Time-Dependent Molecular Remodeling of the Vitreous in Retinal Detachment Reveals a Window of Biological Reversibility

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Abstract

Retinal detachment induces profound biochemical changes within the vitreous humor that evolve over time and critically influence tissue outcome. However, molecular distinctions between early and late stages of detachment remain insufficiently characterized, limiting opportunities for precision diagnostics, prognostic stratification, and timely intervention.

In this study, we performed integrated proteomic, metabolomic, and targeted lipidomic profiling of human vitreous samples obtained from patients with early retinal detachment (≤5 days), late retinal detachment (≥10 days), and control subjects. Using multivariate statistics and pathway-based analyses, we delineated time-dependent molecular signatures reflecting distinct biological states of the detached retina.

Early retinal detachment was characterized by preserved metabolic flexibility and adaptive tissue remodeling. The vitreous proteome showed dynamic extracellular matrix turnover and maintained signaling competence without evidence of acute inflammatory activation. Metabolomic profiling revealed increased levels of uridine, porphobilinogen, and small glycine-rich peptides, indicating active RNA synthesis, mitochondrial engagement, and controlled protein turnover. Lipidomic analysis demonstrated relative depletion of complex phospholipids, consistent with increased membrane turnover and structural plasticity, defining a potentially reversible phase of tissue stress.

In contrast, late retinal detachment displayed a fundamentally different molecular phenotype consistent with chronic maladaptation. Proteomic changes included accumulation of structural extracellular matrix proteins, activation of lysosomal proteases, and markers of low-grade, exhausted immune activity. Metabolically, late detachment was marked by elevated acylcarnitines, docosahexaenoic acid, and gluconate, reflecting impaired mitochondrial fatty acid oxidation and redox imbalance. Lipidomics revealed accumulation of ether-linked phospholipids, plasmalogens, and sphingomyelins, indicating reduced lipid turnover and membrane stabilization.

Our findings demonstrate that retinal detachment is a dynamic, time-dependent process with a definable window of biological reversibility. Vitreous-based multi-omics enables

molecular staging of disease and provides a mechanistic basis for prognostic stratification and precision timing of intervention.

The work was supported from European Regional Development Fund-Project " Interdisciplinary Approaches for the Development and Application of New Materials in Medical Practice - New Omic Technologies " (No. CZ.02.01.01/00/23_021/0009224).

L17

Fast Quantitative Analysis of Selected Oxylipins with the Resolution of Isomers using EAD Fragmentation

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Abstract

Oxylipins are oxygenated derivatives of polyunsaturated fatty acids (PUFAs) that serve as lipid signaling molecules. Due to their dynamic role in physiological and pathological processes, sensitive analysis is required. Selected Reaction Monitoring (SRM) using Collision Induced Dissociation (CID) on Triple Quadrupole (QQQ) based mass spectrometers is typical analysis approach. Some important isomer pairs (for example, 11,12-EET / 11-HETE, or Prostaglandin D_2 / E_2) cannot be fully distinguished with CID, which requires chromatographic separation of these isomer pairs and LC gradients 20 minutes long.

ZenoTOF 8600 delivers similar sensitivity in MRM^{HR} mode like most sensitive QQQ instruments. ZenoTOF is equipped with Electron Activated Dissociation (EAD) that includes Electron Impact Excitation of Ions from Organics (EIEIO) mode, where electrons with the energy of 10 eV attack captured precursor ions providing radical enabled fragmentation [1]. The results are spectra containing significantly more diagnostic fragments than using CID. The presentation highlights how EAD allows distinguishing important isomer pairs of oxylipins and quantifying them using fast LC gradients of 5 minutes.

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L18

SAM Transmethylation Pathway and Adenosine Recycling to ATP Are Essential for Systemic Regulation and Immune Response

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Abstract

During parasitoid wasp infection, activated immune cells of *Drosophila melanogaster* larvae release adenosine to conserve nutrients for immune response. S-adenosylmethionine (SAM) is a methyl group donor for most methylations in the cell and is synthesized from methionine and ATP. After methylation, SAM is converted to S-adenosylhomocysteine, which is further metabolized to adenosine and homocysteine. Here, we show that the SAM transmethylation pathway is up-regulated during immune cell activation and that the adenosine produced by this pathway in immune cells acts as a systemic signal to delay *Drosophila* larval development and ensure sufficient nutrient supply to the immune system. We further show that the up-regulation of the SAM transmethylation pathway and the efficiency of the immune response also depend on the recycling of adenosine back to ATP by adenosine kinase and adenylate kinase. We therefore hypothesize that adenosine may act as a sensitive sensor of the balance between cell activity, represented by the sum of methylation events in the cell, and nutrient supply. If the supply of nutrients is insufficient for a given activity, adenosine may not be effectively recycled back into ATP and may be pushed out of the cell to serve as a signal to demand more nutrients.

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L19

Metabolic Rewiring of Multipotent Mesenchymal Stromal Cells in Three-Dimensional Spheroids

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Abstract

Background: Three-dimensional spheroid culture is a promising strategy to boost the therapeutic potential of multipotent mesenchymal stromal cells (MSCs), yet the metabolic basis of spheroid MSC function remains poorly understood. This study presents an integrative metabolic characterization of human adipose tissue–derived MSCs during spheroid formation and maturation.

Methods: MSCs were cultured as 3D spheroids and analyzed across formation and maturation stages. Cellular bioenergetics were assessed by Seahorse extracellular flux analysis, and global metabolic changes were profiled by untargeted LC–MS-based metabolomics and lipidomics. Growth factor secretion was measured using a Luminex multiplex immunoassay, and spheroid lipidomes were compared with those of their extracellular vehicles (EVs).

Results: Spheroid formation drove progressive cellular compaction, reduced cell size, and increased secretion of pre-regenerative growth factors. Both oxidative phosphorylation and glycolysis were markedly suppressed relative to monolayer cultures, indicating a shift toward a quiescent metabolic state. Metabolomic profiling showed decreased intracellular amino acid levels, consistent with reduced protein synthesis demand. Lipidomic analysis revealed extensive remodeling, including enrichment of diacylglycerols and unsaturated fatty acids, alongside a pronounced reduction in structural phospholipids. Notably, the substantial overlap between spheroid and EV lipidomes supports a role for vesicle-mediated lipid turnover, suggesting that depletion of structural phospholipids may be driven, at least in part, by EV formation.

Conclusions: MSC spheroid formation is accompanied by dynamic metabolic reprogramming, including bioenergetic suppression, altered amino acid utilization, and extensive lipid remodeling. These adaptations are central to spheroid MSC biology and offer mechanistic insight to support the optimization of spheroid-based regenerative therapies.

This work was supported by Czech Science Foundation grants 22-31457S, 26-21666S, and the Ministry of Health of the Czech Republic, grant NW25-10-00263

L20

Phytochemical Composition of Rowanberries

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Abstract

Rowanberry consumption can bring health benefits, as they are rich in bioactive compounds including terpenoids and phenolics. Therefore, the fruit phytochemical composition of ten Czech endemic rowan trees (*Sorbus* L.) was studied, concentrating on fatty acids, terpenoids, and phenolics. Oleic and linoleic acids were the most abundant fatty acids in rowanberries. The profile of fatty acids differed between *Sorbus* species, resulting in varying ratios of polyunsaturated to saturated fatty acids, with the most favorable ratio found in *S. eximia*. Among non-polar terpenoids, β-sitosterol, campesterol, lupeol, and uvaol were identified in the samples. The content of lupeol varied from 24 μg/g DW in *S. eximia* to 78 μg/g DW in *S. omissa* and the content of uvaol varied from 44 μg/g DW in *S. omissa* to 412 μg/g DW in *S. cucullifera*. Among the phenolic compounds, the most abundant were isomers of caffeoylquinic acid, (epi)catechin, and rutin. *S. albensis* was characterized by low contents of caffeoylquinic acids and rutin, *S. eximia* was characterized by a low content of rutin, and *S. cuculifera* was characterized by high contents of (epi)catechin and rutin. It has thus been shown that the fruits of Czech endemic rowan trees exhibit considerable variability in the composition of bioactive compounds.

This work was supported by the Grant Agency of Masaryk University (project No. MUNI/A/1684/2024).

L21

Regioselective and Stereoselective Preparation of Ganaxolone Analogues by Turbo-Grignard Conditions

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Abstract

Neurosteroids are key modulators of central nervous system function, and several synthetic analogues have recently been approved for the treatment of postpartum depression [1]. Their pharmacological activity critically depends on the position and stereochemistry of the C3 hydroxyl group, yet rapid metabolic inactivation limits their efficacy. Alkyl substitution proximal to this site is an established strategy to enhance metabolic stability and prolong bioactivity, as exemplified by ganaxolone.

Here, we report regio- and stereoselective alkylation protocols for neurosteroid scaffolds using turbo Grignard reagents, enabling selective installation of equatorial alkyl groups, as well as additive-controlled formation of axially alkylated hydroxyl derivatives. Guided by molecular modeling, these methods allow prediction of enhanced neuroactivity. In contrast to prior approaches yielding 30–40% [2], our methodology achieves near-quantitative and reproducible conversions.

Furthermore, the strategic incorporation of fluorine atoms at metabolically vulnerable positions is recognized as a powerful approach for fine-tuning pharmacokinetic and pharmacodynamic properties. Accordingly, we extend our methodology to enable the introduction of fluoroalkyl groups, further enhancing therapeutic potential.

This work was supported by the project National Institute for Research of Metabolic and Cardiovascular Diseases (Programme EXCELES, ID Project No. LX22NPO5104) —Funded by the European Union—Next Generation EU and Czech Academy of Sciences institutional support [RVO:67985823] and [RVO:61388963]. Access to computing and storage facilities owned by parties and projects contributing to the Czech National Grid Infrastructure MetaCentrum provided under the programme "Projects of Large Research, Development, and Innovations Infrastructures" (CESNET LM2015042), is appreciated.

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SAGE THERAPEUTICS - WO2020/118060, 2020, A1 Location in patent: Paragraph 000625

L22

timsMetabo™: Uncompromised 4D-Metabolomics™ and 4D-Lipidomics™ at Depth, Speed and Scale

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Abstract

4D-Metabolomics™ workflows are the next step in achieving high confidence in metabolite annotations. Combining the selectivity dimensions of liquid chromatography (LC), trapped ion mobility spectrometry (TIMS) and high-resolution mass spectrometry (HRMS) workflows, the novel timsMetabo instrument achieves unprecedented sensitivity and access to non-chimeric fragment spectra for challenging, fragile metabolites and lipids.

In this presentation, we will discuss the technology enabling small-molecule workflows on timsMetabo and present application examples such as metabolite profiling in complex biosamples, isomer separation for clinical research, and lipid biomarker discovery.

9TH CZECH LIPIDOMICS AND METABOLOMICS CONFERENCE

ABSTRACTS

Posters

P01

Plasma Lipidome Signatures Distinguish Early-Onset Hyperuricemia and Gout and Reflect Response to Urate-Lowering Therapy

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Abstract

Hyperuricemia (HUA) is a key risk factor for gout; however, it remains unclear which patients will progress to clinically manifest disease and how urate-lowering therapy (ULT) affects this process. This study aimed to characterize plasma lipidome alterations in patients with asymptomatic HUA and gout, with particular emphasis on early-onset disease and the impact of ULT.

A total of 343 individuals were included: 94 patients with asymptomatic HUA, 196 gout patients, and 53 normouricemic healthy controls. Lipidomic profiling was performed using LC–MS/MS, enabling semi-quantitative analysis of 608 plasma lipids. Both univariate and multivariate statistical approaches were applied to identify significant differences between groups.

Significant alterations in lipid metabolism were observed in both HUA and gout patients compared to controls. The most prominent changes included upregulation of glycerophospholipids, particularly phosphatidylethanolamines and phosphatidylcholines, and downregulation of lysophosphatidylcholines and their plasmalogen/plasmanyl forms. These changes were more pronounced in patients with early-onset disease (≤40 years), indicating a stronger metabolic disturbance despite generally lower comorbidity burden in younger individuals. ULT had a measurable impact on lipid profiles, particularly in early-onset HUA patients, where treatment shifted lipid composition toward that of healthy controls. This normalization effect was less pronounced in gout patients, indicating possible irreversible or more complex metabolic alterations at later disease stages.

Overall, the findings highlight substantial dysregulation of lipid metabolism in hyperuricemia and gout, especially in early-onset forms. The observed lipidomic signatures may contribute to understanding disease pathogenesis and could serve as potential biomarkers for risk stratification and therapeutic monitoring. Furthermore, the results support the consideration of earlier intervention strategies in selected patients with hyperuricemia.

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P02

Serum Lipid Dysregulation in Systemic Lupus Erythematosus and Lupus Nephritis Indicates Oxidative Stress and Altered Membrane Remodelling

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Abstract

Background: Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterised by chronic inflammation and multi-organ involvement. Lupus nephritis (LN) develops in approximately 50% of SLE patients and leads to kidney damage, significantly contributing to disease morbidity and long-term outcomes. Lipidomics enables comprehensive profiling of lipid species and provides an unbiased means to explore disease-associated alterations. Such approaches may help to identify lipid patterns associated with disease heterogeneity, including differences between SLE patients with and without renal involvement.

Objectives: To characterise serum lipidomic alterations in SLE patients with and without nephritis and to identify lipid patterns associated with disease-related pathophysiological processes.

Methods: Serum samples from SLE patients without nephritis (n = 36), patients with LN (n = 50), and healthy controls (n = 30) were analysed using an MRM-based targeted lipidomic approach based on reversed-phase liquid chromatography coupled with tandem mass spectrometry (RP-LC-MS/MS; Nexera, Shimadzu; QTRAP 6500, Sciex) operating in polarity-switching mode. The method enabled semi-quantitative profiling of more than 900 lipid species. In addition, CERT (Coronary Event Risk Test) scores were determined using a targeted RP-LC-MS/MS method based on internal standard-based absolute quantification or ratio calculation of four ceramides and three phosphatidylcholines. Data were evaluated using univariate and multivariate statistical approaches, complemented by pathway-level analysis (BioPAN).

Results: Lipidomic profiling did not reveal a clear separation between SLE and LN, indicating that disease-specific differences are driven by alterations in specific lipid species rather than global lipid shifts. Both SLE and LN patients exhibited pronounced lipid dysregulation compared to controls. The most prominent changes included decreased levels of ether- and vinyl ether-linked phosphatidylcholines (PC-O, PC-P) and phosphatidylethanolamines (PE-O, PE-P), consistent with impaired lipid homeostasis and increased susceptibility to oxidative stress. In parallel, elevated levels of lysoether phospholipids (LPC-O) pointed to enhanced membrane remodelling. Alterations in polyunsaturated lipid species were characterised by increased circulating polyunsaturated fatty acids and decreased PUFA-containing phosphatidylcholines, suggesting enhanced phospholipase activity. Reduced levels of phosphatidylserines further supported increased membrane turnover and apoptotic signalling. Pathway

analysis confirmed coordinated lipid remodelling, including activation of phosphatidylserine-to-phosphatidylethanolamine conversion, highlighting system-level metabolic adaptation rather than isolated lipid changes. CERT scores (CERT1 and CERT2), reflecting ceramide- and phosphatidylcholine-associated cardiovascular risk, were significantly elevated in both SLE and LN compared to healthy controls, indicating a shift toward a more atherogenic lipid profile. However, CERT scores did not clearly distinguish between SLE and LN.

Conclusion: Serum lipidomic alterations in SLE and LN indicate coordinated changes in lipid metabolism associated with oxidative stress and membrane remodelling. While CERT scores reflect global cardiometabolic alterations, lipidomic profiling provides additional resolution at the level of specific lipid pathways. These findings contribute to a better understanding of disease-associated lipid patterns in SLE with and without renal involvement.

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P03

Orally Administered Hyaluronan Exerts Metabolic Effects Distinct from Pectin in Healthy Mice

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Abstract

Orally administered hyaluronan (p.o. HA) has been reported to exert extra-intestinal beneficial effects across multiple clinical contexts, including joint function, inflammatory status, skin aging, and metabolic health, yet the mechanisms underlying these outcomes remain unclear and are likely indirect. In this context, it is also important to determine whether HA acts through mechanisms distinct from those of other soluble dietary fibers, such as pectin. In this study, we have investigated the orally administered HMW-HA (1.7 MDa) and pectin (0.25 MDa) roles on healthy C57/BL/6 female mice (n=30, 10 per group) after 14 days of treatment. Lipidomics and metabolomics of the liver and plasma were performed. Specific HA effects in comparison with pectin were observed mainly in the liver metabolome, with potential antioxidant and energy metabolism effects. Both plasma and liver lipidomics revealed specific and correlated HA effects with potential immunomodulatory effects driven by altered lysophospholipids. The results bring new insights into the HA systematic function mechanism and suggest that the HA effect is different from general fibers.

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P04

An Automated Python Workflow for End-to-end Processing and Statistical Analysis of Targeted Metabolomics Data from SciexOS

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Abstract

Targeted LC–MS/MS metabolomics experiments generate complex datasets requiring extensive preprocessing, quality control, and statistical analysis prior to biological interpretation. These steps are often performed using multiple software tools and manual interventions, which can reduce reproducibility and hinder standardization. To address this, we developed a comprehensive Python-based workflow for automated, end-to-end processing of data exported directly from SciexOS.

The workflow performs systematic data curation, including restructuring of input tables and removal of analytes marked as not-detected. Quality control procedures include filtering of analytes with excessive signal in blank samples, exclusion of samples failing predefined criteria for injection sequence performance, and removal of features with high proportions of missing values. Additional filtering based on coefficient of variation (CV) in quality control (QC) samples ensures analytical robustness.

Signal normalization is performed using internal standards, with additional pattern-based handling implemented for lipid classes. Analytical drift across the batch is corrected using LOESS regression. Remaining missing values are imputed, followed by data transformation and scaling to prepare datasets for statistical analysis.

The statistical module integrates both univariate and multivariate approaches. Univariate analysis includes normality testing, outlier detection, descriptive statistics, and hypothesis testing, accompanied by visualization tools such as boxplots and interactive volcano plots. Multivariate analysis includes principal component analysis (PCA) and hierarchical clustering, with outputs such as dendrograms and heatmaps. The workflow further supports network-based visualization and receiver operating characteristic (ROC) analysis for evaluation of discriminatory performance.

This Python workflow provides a flexible, reproducible, and scalable solution for targeted metabolomics data processing. By integrating preprocessing, quality control, and statistical analysis into a single framework, it reduces manual intervention and facilitates standardized, high-throughput data analysis.

P05

Metabolomic Profiling of Honey Bee Hemolymph by LC–MS/MS Reveals Diet-dependent Metabolic Differences

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Abstract

Introduction: The honey bee (Apis mellifera) is a key pollinator species whose health, development, and colony performance are strongly influenced by nutrition, particularly the intake of carbohydrates, amino acids, and other essential nutrients1,2. Hemolymph, the main circulatory fluid of insects, plays a crucial role in nutrient transport, energy metabolism, and immune responses, and therefore represents a sensitive indicator of the physiological state of the organism3,4. Its composition reflects both internal metabolic processes and external factors, including diet. Therefore, hemolymph represents a highly suitable biological matrix for metabolomic analysis, enabling comprehensive characterization of physiological changes associated with nutritional conditions in honey bees.

Methods: A total of 144 hemolymph samples were analyzed, divided into four dietary groups: pollen, UltraBee supplement, chlorela, and sugar solution (control group). LC–MS/MS analysis was performed using a Nexera™ LC system coupled with a HILIC Luna NH_2 column (3 µm, 100 × 2 mm, Phenomenex) and a QTRAP® 6500+ mass spectrometer (SCIEX), operating in MRM mode with polarity switching. Targeted metabolomic profiling focused on amino acids, acylcarnitines, sugars, nucleotides, and selected intermediates of central metabolism. Statistical evaluation involved univariate and multivariate analyses to assess differences between dietary groups.

Results: Preliminary results revealed clear diet-dependent differences in honey bee hemolymph metabolomic profiles. The sugar-fed control group showed the most pronounced deviation, while pollen-, chlorella-, and supplement-fed bees exhibited more comparable profiles, consistent with a more balanced nutritional status. These differences were mainly driven by changes in selected metabolites, particularly amino acids and long-chain acylcarnitines, indicating alterations in key metabolic pathways related to energy metabolism and nutrient utilization.

Conclusion: This study demonstrates that LC–MS/MS-based metabolomics enables sensitive detection of diet-dependent metabolic changes in honey bee hemolymph. Nutritionally imbalanced diets led to distinct metabolic alterations, whereas more complex diets supported a more stable metabolic profile. Identified metabolomic signatures, especially amino acids and acylcarnitines, may serve as potential biomarkers of nutritional status and bee health, although their biological roles require further investigation.

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P06

ViLiFOG: Automating the Construction of Lipidomic Models for Metabolic Flux Analysis

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Abstract

Metabolic flux analysis (MFA) allows scientists to quantitatively describe cellular metabolism by calculating flux values of individual metabolic reactions. Such information can be useful to determine dysregulated pathways which may, for example, help elucidate mechanisms underlying various diseases. In order to conduct MFA, dedicated software has to be provided with a metabolic model, i.e. a set of metabolic reactions whose flux shall be calculated. To produce a list of reactions might not be a difficult task in case of simple pathways such as glycolysis or Krebs cycle but in case of lipidomic pathways, the situation is fundamentally different. Lipidome consists of various lipid groups, each containing multiple individual lipid species making it one of the most diverse metabolite groups. Moreover, many enzymes exist, carrying out conversions between different lipid groups. That makes the number of unique reactions grow polynomially, rendering it unfeasible to define all of them manually.

To address this issue, Python programming language was used to develop Virtual Lipidome Flux Object Generator (ViLiFOG). With ViLiFOG, one can have the lipidomic model defined automatically. Based on the user selection of fatty acids and available enzymes, ViLiFOG generates all the lipid species and reactions taking place among them, including atom mapping. The application's output is directly compatible with INCA, a software dedicated to MFA calculation, to ensure the smoothest transition between the automatically generated model and meaningful flux values.

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P07

Lipidomic Signatures of Subcutaneous *De Novo* Lipogenesis Track with Ectopic Fat Dynamics during Dietary Carbohydrate Manipulation

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Abstract

Background: De novo lipogenesis (DNL) in subcutaneous adipose tissue (SAT) is downregulated, while accumulation of ectopic fat, particularly in liver and muscle is enhanced in obesity. Aim of our study was to investigate the human lipidomic signatures of SAT DNL and its association with ectopic fat accumulation. By utilizing an isocaloric ketogenic diet (KD), we created a physiological framework to study DNL shifts under conditions of variable glucose availability.

Methods: In this study, twenty-two obese women (age 36 ± 6 years, BMI: 36.1 ± 3.8 kg/m^2) successfully completed a 28-day KD intervention (8% saccharides) followed by a 2-day carbohydrate refeeding period (60-65% saccharides). Abdominal SAT biopsies were collected, and a comprehensive multi-omics strategy was employed, combining SAT proteomic and lipidomic analyses with magnetic resonance-based quantification of hepatic fat content and intramyocellular lipids (IMCL).

Results: The KD induced a profound suppression of key SAT DNL proteins (FASN, ACACA, SCD1). Because their levels consistently tracked with DNL protein abundance across all phases of the diet, specific short-chain triglycerides (TAG 38:1, 40:1, 40:2, 42:2, and 42:3) and phosphatidylinositols (PI 34:2, 36:1, 36:2, 36:3) were identified as surrogate markers of SAT DNL. These “DNL-TAGs” correlated negatively with HFC in all three phases. Relative changes in DNL-TAGs correlated negatively with changes in IMCL during KD.

Conclusion: Our findings demonstrate that KD-induced suppression of SAT DNL is accurately reflected by a specific lipidomic signature. The negative correlation between these DNL-markers and ectopic fat depots (HFC and IMCL) suggests that SAT DNL activity may be a key determinant of systemic lipid partitioning. These results highlight the potential of "DNL-TAGs" as clinical indicators of metabolic health.

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P08

Analysis of the Impact of Anesthesia on Metabolic Changes Using a $^{13}C_6$-Glucose Tracer in a Mouse Model

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Abstract

Anesthesia is a common part of experiments involving laboratory animals; however, it may influence metabolism and confound experimental results. The main issue is that the metabolic effects of anesthetics are still not well characterized. The aim of this project is to compare the impact of two anesthetics, (Isoflurane and Zoletil/Xylazine), on metabolic and especially lipidomic profiles in *C57BL/6* mice (*Mus musculus*) using ^{13}C-labeled glucose tracing. Animals will be administered ^{13}C-labeled glucose intraperitoneally, followed by anesthesia. After 30 minutes, animals will be sacrificed, and tissue and plasma samples will be collected. The control group will comprise animals sacrificed by cervical dislocation. The results of this study may contribute to a better understanding of anesthesia-induced metabolic changes and facilitate the selection of suitable anesthetics for specific experimental designs.

P09

Relatively High Metabolic Flexibility of Epicardial Adipose Tissue Metabolism in Cardiac Cachexia

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Abstract

Cardiovascular disease remains the leading global cause of mortality, with heart failure (HF) accounting for most deaths. Approximately 10% of HF patients develop cardiac cachexia, a severe metabolic syndrome that markedly worsens prognosis. In our previous study of epicardial adipose tissue (EAT) in advanced HF, we identified distinct metabolic and molecular signatures associated with cachexia [1]. Cachectic patients exhibited elevated plasma BNP levels, altered natriuretic peptide receptor expression favouring lipolysis, reduced EAT thickness, and profound shifts in the EAT metabolome, most notably an accumulation of the mitochondrial phospholipid cardiolipin 70:6. These findings suggested that BNP-driven lipolysis and cardiolipin remodeling may contribute to adipose tissue wasting. Building on these observations, the present study investigated how cachexia alters metabolic interactions between EAT and myocardium (MYO).

We first examined metabo-lipidome co-expression profiles in paired EAT and MYO samples from 50 patients using WGCNA method. To account for inter-individual variability in tissue lipid content, all analyte concentrations were normalized to tissue DNA content. A subset of BW-stable (n=9) and cachectic (n=9) individuals from the original cohort was selected for integrated transcriptomic, metabo-lipidomic, and proteomic analyses (proteomics in MYO only). In EAT, DNA content correlated positively with BW loss, indicating adipocyte shrinkage due to lipid depletion. In MYO, DNA content remained relatively constant, but triglyceride (TAG) levels—particularly long-chain TAG species—were ten-fold higher in BW-stable compared with cachectic patients. The selective loss of long-chain TAGs in MYO suggests accelerated TAG turnover in cachexia, with breakdown exceeding synthesis. Together, reduced EAT lipid stores and diminished MYO TAG content point to substantial remodeling of the EAT–MYO metabolic axis, consistent with reduced lipid availability for myocardial oxidation.

To further dissect tissue-specific responses, we applied supervised multi-omics integration using the DIABLO framework, which extends multi-block PLS-DA to identify components that best discriminate BW-stable and cachectic patients. A model with three components per omics block was fitted. In EAT, group separation was driven primarily by component 1, whereas in MYO it emerged on component 2, indicating that cachexia-related variation is more pronounced in EAT. Feature loadings revealed clear tissue-specific patterns: EAT displayed a coherent metabolic response dominated by cardiolipin remodeling, enhanced lipolysis, and signatures of elevated fatty acid

oxidation. In contrast, MYO separation was associated mainly with pathways related to intracellular signaling, structural remodeling, and stress responses rather than core metabolic pathways.

In summary, cardiac cachexia induces stronger and more uniform metabolic alterations in EAT than in MYO, underscoring a primary role for EAT in shaping the metabolic microenvironment of the failing heart.

This work was supported by the Ministry of Health (AZV ČR NW26-02-00105).

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P10

Targeted Metabolomic Profiling Reveals Differentiation-Dependent Effects of Methadone in SH-SY5Y Neuroblastoma Cell Line

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Abstract

The SH-SY5Y cell line both undifferentiated and differentiated is one of the most widely used *in vitro* models in neuroscience research. The differentiation shifts the neuroblastoma cells into neuronal-like phenotypes and significantly changes its biochemical properties, including changes in neurotransmitter systems, but further investigation of these changes is still lacking, even though a comprehensive understanding of the activity of neurotransmitter signaling pathways is key to selecting the right *in vitro* model for studying neuroactive substances. Methadone, a synthetic opioid used in pain management and opioid substitution therapy, exhibits pleiotropic effects on multiple neurotransmitter systems. The differentiation status of SH-SY5Y cells can thus significantly influence the effects of this substance. The aim of this study was to quantify the major neurotransmitters in SH-SY5Y cells before and after differentiation and to compare the effect of methadone on these cellular phenotypes. We found that the differentiation of SH-SY5Y cells lowers the level of amino acids, polyamines, cAMP and serotonin and alters the level of catecholamines and acetylcholine. In undifferentiated cells administered to methadone higher levels of glutamate, GABA, glutamine, glycine and kynurenine were observed. Methadone also increased the level of adenosine in differentiated cells. These findings suggest that differentiation status strongly influences neurotransmitter signaling pathways and modulates the cellular response to methadone, highlighting the importance of appropriate *in vitro* model selection in neuroscience research.

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P11

Integrating Metabolomics and Functional Traits to Uncover Tissue-Specific Responses to Novel Climates in a Grassland Species

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Abstract

Grassland ecosystems play a crucial role in global carbon cycling and biodiversity maintenance. However, predicting their responses to future climate remains challenging due to a lack of mechanistic knowledge linking interacting environmental drivers to plant adaptive strategies. Most studies infer plant responses from physio-morphological traits or examine individual climate drivers in isolation, whereas evidence integrating metabolic processes under multi-factor climate in wild species remains scarce. Our study addresses these gaps by characterizing the metabolomic and physio-morphological responses of Plantago lanceolata to decade-long multi-factor climate manipulation in the Austrian Alps. Within this ClimGrass experiment, plants were grown under simulated future climatic regimes, which combined warming, recurrent summer drought, and elevated CO_2. We collected leaf and root samples from 24 field plots representing six climatic regimes, with four replicate plots per treatment. Physio-morphological traits, including chlorophyll content, stomatal conductance, specific leaf area, leaf dry matter content, specific root length, and root tissue density, were quantified to characterize functional responses. In parallel, untargeted metabolomic profiling using liquid chromatography–quadrupole time-of-flight mass spectrometry (LC–MS QTOF) will capture regime-specific shifts in primary and secondary metabolism, including phenylpropanoids, flavonoids, and terpenoid-derived compounds, across above- and belowground tissues. The study addresses three key questions: (i) how warming, drought, and elevated CO_2 individually and interactively influence metabolomic profiles in leaves and roots; (ii) whether metabolomic variation aligns with shifts in functional traits; and (iii) whether above- and belowground tissues differ in their sensitivity to multi-factor climate manipulation. We hypothesize that combined climatic factors induce non-additive, tissue-specific metabolic reprogramming; with roots exhibiting stronger shifts in metabolites associated with osmotic regulation and stress tolerance, and leaves showing adjustments linked to carbon assimilation and defense-related pathways. The findings will emphasize the complex and integrative nature of plant metabolic and physio-morphological responses to novel climates, highlighting the importance of multi-factor climate interactions for predicting plant responses to future climates.

P12

Analysis of Triacylglycerol Estolide Stereoisomers by Chiral SFC-MS

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Abstract

Triacylglycerol estolides (TG EST) represent a class of bioactive lipids whose metabolic roles remain poorly understood. Their analysis is challenged by extensive structural diversity, limited availability of reference standards, and the lack of stereoselective analytical methods. This study aims to evaluate the applicability of chiral supercritical fluid chromatography–mass spectrometry (SFC–MS) for the separation of TG EST stereoisomers and regioisomers. In this study, nine TG EST standards containing fatty acid ester of hydroxy fatty acid (FAHFA) were systematically investigated using chiral SFC-MS with various polysaccharide-based stationary phases. Key chromatographic parameters, including mobile phase composition, flow rate, temperature, and backpressure, were systematically optimized. Two amylose-based columns connected in series provided the best resolution of TG EST stereoisomers. In addition, a reversed-phase liquid chromatography (RP-LC) method was developed to resolve regioisomers. The combination of chiral SFC–MS and RP-LC revealed a high structural complexity of TG EST species annotated in white adipose tissue samples. The developed methodology provides a novel tool for the stereoselective analysis of TG EST and may contribute to a deeper understanding of their biological functions.

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P13

What Do We Really Measure? Impact of Sample Preparation on the Biological Interpretation of Fatty Acid Profiles

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Abstract

Fatty acid (FA) profiling is widely applied in clinical and biochemical studies, particularly in plasma analysis, where it is used to assess metabolic status and nutritional influences. However, the obtained FA composition strongly depends on the sample preparation strategy, which determines the lipid pool being analysed.

In this study, we evaluated the effect of different extraction, fractionation, and derivatization approaches on the resulting FA profiles. Lipids were isolated using the modified Folch extraction (chloroform–methanol), followed by liquid–liquid partitioning into polar and non-polar phases. To further investigate lipid class selectivity, solid-phase extraction (SPE) using aminopropyl sorbent was applied. Subsequently, two derivatization methods were compared: base-catalyzed transesterification targeting predominantly esterified fatty acids, and acid-catalyzed methanolysis providing total FA content, including free fatty acids.

The results revealed that each step of the analytical workflow significantly affects the measured FA composition. It was observed that lipid-bound FA are not exclusively present in a single phase, suggesting limited selectivity of lipid class partitioning during extraction. SPE fractionation further indicated that lipid classes are not uniformly separated, pointing to incomplete selectivity of the fractionation process. Furthermore, substantial differences between derivatization approaches confirmed that distinct analytical strategies reflect different fatty acid pools.

These findings demonstrate that FA profiles obtained by different analytical workflows are not directly comparable and represent different aspects of lipid metabolism. In particular, total FA analysis includes contributions from free fatty acids, which may reflect circulating and more dynamic lipid pools, whereas analysis of esterified fatty acids predominantly represents structural and storage lipid pools. Therefore, sample preparation should be considered a critical factor determining not only analytical outcomes but also the biological and clinical interpretation of FA data.

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P14

Application of mCPBA Derivatization for Structural Analysis of Unsaturated Lipids

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Abstract

Localization of double bonds in unsaturated lipids remains an important challenge in lipidomics. Derivatization with meta-chloroperoxybenzoic acid (mCPBA) enables selective epoxidation of carbon–carbon double bonds and produces characteristic MS/MS fragmentation useful for structural identification. In this work, phosphatidylcholine PC 36:2 is presented as a model system to demonstrate how mCPBA derivatization combined with mass spectrometry analysis can be used to determine fatty acid composition and double bond positions. This approach improves the structural characterization of lipid isomers in complex samples.

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P15

Evaluation of SPE Strategies for Targeted and Untargeted LC-MS Metabolomic Analysis

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Abstract

Sample preparation remains one of the most critical and challenging steps in liquid chromatography – mass spectrometry (LC-MS) metabolomic analysis, as it directly affects metabolite recovery and analytical accuracy and reliability. In this study, we systematically compared solid-phase extraction (SPE) strategies for serum sample preparation in both targeted and untargeted metabolomic approaches. Metabolites with diverse structural and polarity characteristics were analyzed using various SPE formats, including dispersive SPE with multiple sorbents and extraction modes, SPE spin columns, SPE pipette tips, and conventional SPE cartridges. Sorbents within the same extraction mode yielded comparable results. Hydrophilic interaction liquid chromatography sorbents demonstrated the highest performance across a wide range of polarities, whereas reversed-phase sorbents favored moderately polar compounds. Ion-exchange sorbents exhibited limited suitability for broad metabolite coverage due to strong pH dependence, but improved recovery of ionizable compounds when combined with other sorbents. While different SPE formats showed similar extraction efficiency, their repeatability varied, with spin columns outperforming conventional cartridges. SPE exhibited mitigation of matrix effects in targeted analysis, particularly for highly polar metabolites, compared to protein precipitation (PPT). Although PPT offered higher efficiency in the untargeted workflow, SPE increased feature coverage by up to 50%. Among commercial products, hydrophilic-lipophilic balanced (HLB) sorbents delivered superior efficiency and reproducibility, with HLB-packed spin columns providing the most universal and robust performance for LC-MS metabolomic analysis.

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P16

Bioinformatic Approaches for Evaluation of LC-MS Metabolic Changes in Biological Studies

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Abstract

In biological research, observed outcomes are often determined by the interplay of multiple factors, yet most studies focus primarily on the direct effects of key variables. A comprehensive understanding, however, requires consideration of secondary influences as well as careful control of pre-analytical variability. In this study, we employed a reversed-phase liquid chromatography–mass spectrometry (RP-LC/MS) platform [1] to characterize metabolic profiles in a well-defined cohort comprising both treated and control groups. Multivariate biostatistical methods were applied to identify key metabolic alterations distinguishing experimental conditions. Beyond the identification of primary differences, we focused on the characterization of secondary effects, including their contribution to overall variability within the dataset. Correlation analyses and regression-based approaches were used to disentangle complex relationships and reveal subtle patterns not captured by direct comparisons. This integrative framework enables a more comprehensive interpretation of metabolic data by accounting for both primary and secondary sources of variation.

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P17

Identification of Cholesterol Binding Sites on the M_1 Muscarinic Acetylcholine Receptor by Rationally Designed Steroid-dark Quencher Probes

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Abstract

The membrane cholesterol was found to bind and modulate the function of several GPCRs including muscarinic acetylcholine receptors. [1] Recently, we have shown that neurosteroids and steroid hormones can act as allosteric modulators of muscarinic receptors and interact with the receptor cholesterol-binding site. [2,3] We aim to locate the cholesterol-binding site and develop selective allosteric modulators based on the sterol structure, offering a new approach for pharmacological targeting of muscarinic receptors and other GPCRs. To identify the binding sites of neurosteroids at muscarinic receptors, we have established a fluorescence quenching-based method. Fluorescence quenching between a fluorescent protein fused to the M_1 receptor at the C and N-terminus (donor) and a dark quencher conjugated with a steroid (acceptor) was utilized to detect the binding of steroids. First, neurosteroids with high affinities were identified and tagged with a dark quencher. Further structure-activity relationship study of the steroid-quencher conjugates allowed us to identify steroid probes with affinities in the hundreds of nanomolar range for the M_1 receptor. Molecular docking and MD simulations identified key residues at the N- and C-terminal sites mediating binding. Competition experiments with non-quenching analogues validated the specificity of the probes. The development of selective allosteric modulators targeting the cholesterol-binding site represents a new avenue of GPCR drug development.

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P18

Multi-omic Signature of Human Plasma After Acute Coronary Syndrome and Stroke

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Abstract

Alterations in lipid metabolism mediated by oxidative stress play a key role in atherosclerosis and superimposed thrombosis, pathologies that may lead to acute coronary syndrome (ACS) and acute ischemic stroke (AIS), respectively. In this study, plasma lipid and protein fingerprints from patients with ACS or AIS were compared with those of controls to discover underlying pathways affected by these events.

Untargeted lipidomics was performed using reverse-phase liquid chromatography coupled to an Orbitrap mass spectrometer, with lipid identification based on $ddMS^2$, spectra matched against in silico libraries. Proteomics analysis employed nanoLC coupled to an ion-mobility QTOF mass spectrometer, and protein identification was carried out using the DIA-NN software.

Lipidomics data were processed using a combination of univariate and multivariate statistical analyses to identify cohort-specific molecular patterns. In total 409 lipids were detected in plasma, 22 of them mainly from the LPC, LPE, and triacylglycerol classes, showed the strongest discriminatory power. Despite very similar lipidomic plasma profiles, proteomics demonstrated superior classification performance, with 526 out of 4357 proteins significantly different among the studied groups. Major contributors to group separation included triglyceride lipase and several creatine kinases.

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P19

Activity of Steroids with Atypical Stereochemistry on Ion-channel Receptor

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Abstract

Neurosteroids constitute a distinct group of endogenous biologically active compounds that play a central role in brain function and homeostasis. Unlike classical steroid hormones, their primary mode of action involves direct modulation of ligand-gated ion channels, such as gamma-amino-butyric acid ($GABA_A$) and *N*-methyl-*D*-aspartate (NMDA) receptors.[1,2] This mechanism underlies their rapid effects on neuronal signaling and links them to key physiological and pathological processes, including synaptic plasticity, cognition, neurodegeneration, and neuropsychiatric disorders.[3-5] Despite their biological relevance, the rational design of neurosteroid-based modulators remains difficult, largely due to the limited understanding of structure-activity relationships and lack of information about binding sites.

In the current study, we explore both how three-dimensional architecture and substituents in key positions govern the activity of steroidal systems. Even subtle changes in the configuration of stereogenic centers could significantly reshape the steroid skeleton, and we hypothesized that this could alter receptor interactions. To test this, we targeted positions that are rarely encountered in natural steroids, introducing atypical stereochemical arrangements.

A synthetic route starting from commercially available dehydroepiandrosterone was developed to access a series of structurally diverse steroid analogues with atypical skeletal shapes and variations in the main functional groups. The resulting compounds were structurally characterized, and their conformations were confirmed by X-ray crystallography. The synthesized library was further evaluated in terms of physicochemical and pharmacokinetic-relevant properties, including stability, solubility, and permeability. Functional assessment at NMDA receptors identified hit molecules, highlighting the impact of stereochemical modifications on biological response. Additionally, a novel approach to predict the biological activity of our steroid molecules was tested based on biological data on the NMDA receptor.

The results of our study emphasize the importance of stereochemical design in shaping neurosteroids' function and introduce alternative types of steroid skeletons that expand the accessible chemical space for the development of novel neurosteroid drug-like compounds.

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P20

Laboratory of Metabolomics: Services and Analytical Platforms

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Abstract

The Laboratory of Metabolomics at the Institute of Physiology of the Czech Academy of Sciences (https://metabolomics.fgu.cas.cz/) provides advanced liquid chromatography–mass spectrometry (LC–MS)-based metabolomics and lipidomics analyses for the identification and quantification of polar metabolites and complex lipids in biological samples. The laboratory supports biomarker discovery and mechanistic studies related to cardiometabolic and other diseases.

Using the in-house developed LIMeX (LIpids, Metabolites, and eXposome compounds) workflow, the laboratory performs comprehensive analyses of plasma, serum, urine, feces, tissues, and cells [1–3]. The workflow combines biphasic extraction with multiplatform LC–MS to maximize metabolite coverage while requiring only small sample amounts. Data processing and compound annotation are performed using MS-DIAL together with in-house, open-source, and commercial MS/MS spectral libraries.

Depending on sample type and study size, the workflow typically reports 500–800 complex lipids and 100–200 polar metabolites. Processed datasets are further analyzed using in-house bioinformatics pipelines to generate standardized statistical outputs, including pathway and overrepresentation analyses.

The laboratory also offers targeted analyses of lipid mediators, drugs and their metabolites, short-chain fatty acids, and fatty acid profiling.

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