4th International Lipidomics Society Conference

MADRID Spain, 28-31 Oct **2025**
Hospital Universitario Ramón y Cajal-IRYCIS

Table of contents

To access the communication quickly, click on the title.

ccl_4 mouse model of liver fibrosis

35. Unveiling cellular changes in leukaemia cell lines after cannabidiol treatment through lipidomics
36. Adaptive lipidomic responses of the marine fungus zalerion maritima to environmental stressors: a promising source of bioactive lipids
37. Exploring lipidome in fragile x syndrome
38. Lipid biomarker discovery in plasma of patients with heart failure and type 2 diabetes undergoing metabolic correction with tirzepatide
39. Lipid profile of metabolic dysfunction-associated steatotic liver disease (masld) among people living with hiv after hcv eradication
40. Enhancing confidence annotation in lipidomics by implementing rule-based annotation and retention time modelling in lipidms
41. Lipidms shiny app: improving lipid annotations through retention time modeling in lipidomics
42. Lipid signatures in urinary extracellular vesicles as non-invasive biomarkers in alcohol use disorder
43. Comprehensive lipidomic characterization of livers in app/ps1 transgenic mice: comparison of profiles between treated and untreated mice
44. Fattening up the facts: how diet can improve lamb muscle lipid profiles
45. Detection limits of oxidized bulk phospholipids in biological samples using conventional matrices and 1-pyrenebutyric hydrazide in maldi-tof ms
46. Comprehensive lipidomic analysis of human blood by ultrahigh- performance supercritical fluid chromatography-mass spectrometry
47. Multiplatform high-resolution metabolomics and lipidomics reveals etiology specific biomarkers in community acquired pneumonia
48. Aso-mediated inhibition of degs1 aggravates inflammation and hepatic fibrosis in mice by increasing free cholesterol storage
49. Transcriptomic and lipidomic insights into lipid metabolism dysregulation in elovl1-deficient fibroblasts
50. Exploring skin lipidomics and lipid delivery with desi-xs
51. Potential role of very long chain fatty acids in the development of early vascular ageing
52. Simultaneous characterization of queen bee-head lipids and pheromones using 4d-lipidomics
53. Untargeted lipidomics identifies pleiotrophin as a regulator of hepatic lipid metabolism with sex-specific effects in a mouse model of diet-induced obesity
54. Integrated multi-omics analysis of barley responses to combined drought and fusarium pseudograminearum infection
55. The synthetic trpml1 agonist ml-sa1 reduces intracellular lipid accumulation by promoting the release of extracellular microvesicles via increased ceramide synthesis de novo
56. Uncovering the effects of air pollution on lung health using human bronchial epithelial cells and organoid models
57. Effects of air pollutants on skin health: from keratinocyte toxicity to lipidomic remodeling in reconstructed skin
58. Lipidomic profiling to monitor phytoceramide biosynthesis in engineered saccharomyces cerevisiae
59. Evaluation of tribrid orbitrap mass spectrometer for structural lipidomics of primary cilia
60. Uncovering lipid signatures of inflammatory cholestasis through rp-lc-ms
61. Metabolomic and lipidomic profiling identifies novel plasma signatures in patients with transthyretin cardiac amyloidosis and aortic valve stenosis diseases
62. Plasma lipidomics signature reveals differential glucose use in idiopathic thoracic aortic aneurysm in patients with tricuspid and bicuspid aortic valves
63. Deep lipidome coverage with high confidence annotations using orbitrap astral zoom ms
64. Searching for fahfa transporters in blood
65. Adipose tissue is the source of ketone bodies in advanced heart failure: direct analysis of fat metabolic remodeling
66. Effect of 6-month nutritional and lifestyle intervention on oxylipines and inflammatory markers in masld patients.
67. Targeted lipidomics reveals systemic effects of pectin intervention in ltp-allergic patients.
68. Lipidomic profiling reveals altered fatty acid and carnitine metabolism in cofactor-induced ltp anaphylaxis
69. Increased serum bile acid concentrations in obese patients after bariatric surgery.

70. Investigating the relationship between guselkumab treatment and signaling lipid levels in blister and plasma samples from psoriasis patients
71. A lipidomic exploration of the effects of high-intensity interval exercise in healthy men after metformin intake
72. Feasibility of identifying gallbladder cancer by simple and direct analysis of human bile using paper spray ionization mass spectrometry
73. Quantitation of lipids via phosphorous using inductively coupled plasma mass spectrometry with oxygen reaction gas.
74. Spatial multi-omics reveal lipid biomarkers of tumor heterogeneity in colorectal cancer
75. Dietary radish bioactives and their impact on brain lipidome remodeling and oxysterol homeostasis in obesity
76. The impact on human plasma lipidome of patients with colon cancer is stage- and sex-dependent: specific decrease on linoleic acid-containing species
77. Development of an automated lipidomics system for reliable data accumulation toward molecular precision medicine
78. Small molecule omics analysis using the zenotof 8600 system
79. Impact of dietary supplementation with docosahexaenoic acid in regio- and enantiopure triacylglycerols on metabolomic profiles of liver and brain in rats
80. Malassezia-mediated microbe/host interaction: clinical insights via oxylipin lipidomics
81. Untargeted lipidomic analysis of liver samples: changes in a mouse model of anaphylaxis

Towards global glycosphingolipidomics using advanced high-resolution mass spectrometry and automated annotation workflows

Amirreza Dowlati Beirami (1), Katharina Hohenwallner (1), Leonida Lamp (2), Petra Heffeter (3), Walter Berger (3), Dominik Egger (4), Ursula Hiden (5), Gavin Reid (6), Jürgen Hartler (2), Evelyn Rampler (1)

(1) Department of Analytical Chemistry, Faculty of Chemistry, University of Vienna, 1090 Vienna, Austria., (2) Institute of Pharmaceutical Sciences, University of Graz, 8010 Graz, Austria., (3) enter for Cancer Research and Comprehensive Cancer Center, Department of Medicine I, Medical University Vienna, 1090 Vienna, Austria., (4) Biofabrication for Drug Testing, Leibniz University Hannover, Hannover, Germany, (5) University of Graz, Institute of Pharmaceutical Sciences, Graz, Austria, (6) School of Chemistry, University of Melbourne, Parkville, Victoria 3010, Australia.

Type of abstract: ORAL

Glycosphingolipids (GSLs) are critical for cell-cell communication, growth, host-pathogen interactions, and signal transduction. They expand the diversity of the lipidome far beyond what conventional lipidomics assays typically capture, which tend to focus on bulk lipid classes. However, glycosphingolipidomics remains an emerging field due to the limited availability of commercial standards and comprehensive methods for measuring and identifying native GSLs. Mass spectrometry (MS) enables the simultaneous monitoring of hundreds of glycosphingolipid species with distinct lipid compositions, distinguishable by their mass-to-charge ratios and fragmentation patterns. High-resolution MS (HRMS) offers particular advantages: high mass accuracy, reduced spectral complexity, and minimized false annotations.

To enhance structural resolution, we developed HRMS-based strategies that integrate: (1) one- and two-dimensional liquid chromatography (Vanquish, Thermo; Agilent Infinity 1290 2D system), (2) field asymmetric ion mobility spectrometry (FAIMS), and (3) advanced fragmentation techniques, including MS^n, ultraviolet photodissociation (UVPD, Orbitrap Fusion Lumos with 213 nm laser), and electron-activated dissociation (EAD, ZenoTOF 7600, Sciex). These approaches allow detailed molecular-level characterization of glycan headgroups, glycolipid classes, ceramides, and fatty acid compositions.Adding an extra separation dimension–via LC or ion mobility–was essential to address the extreme chemical complexity of GSLs. For example, gangliosides could be separated by class and charge state based on their glycan headgroup. The more sialic acids present, the higher the charge state observed: trisialylated gangliosides mainly appeared as $[M-3H]^{3-}$, while $[M-4H]^{4-}$ and $[M-5H]^{5-}$ ions were detected for GQ1 and GP1, respectively[1]. Fragmentation was essential for resolving GSLs down to the molecular lipid species level. To scale this toward global glycosphingolipid analysis, we extended our 30-minute reversed-phase HRMS workflow[2] to distinguish acidic and neutral GSLs in sensitive and resistant colorectal cancer cell lines treated with oxaliplatin and BOLD-100, revealing glycosphingolipid upregulation in BOLD-100-treated cells. Currently, we are evaluating an online 2D-LC approach combining hydrophilic interaction chromatography (HILIC) and reversed-phase (RP) chromatography, coupled with HRMS, to monitor structural changes in intact GSLs in resistant and sensitive cancer samples and during mesenchymal stem cell differentiation.

Despite advances in mass spectrometry, many GSL workflows still rely on manual annotation, making the combinatorial complexity of GSLs a major bottleneck for global glycosphingolipidomics. To address this, we developed FraGLI, an extension of the Lipid Data Analyzer[3] software, which supports the automated generation of mass lists and decision rules for identifying known and predicted GSL classes. Using our non-targeted workflow, we confirmed 19 acidic and neutral GSL classes and several hundred unique molecular species in GSL standards and colorectal cancer samples. Notably, we observed significant changes in the overall GSL profile under two different drug-resistant conditions.

While the success of global glycosphingolipidomics is inherently sample-type, extraction, and measurement dependent, we believe that combining advanced high-resolution MS with automated annotation tools like FraGLi offers the community a powerful tool for truly global GSL analysis.

References

[1] [1] K. Hohenwallner, L. M. Lamp, L. Peng, M. Nuske, J. Hartler, G. E. Reid, E. Rampler, Anal. Chem. 2024, DOI 10.1021/acs.analchem.4c01313.
[2] [2] K. Hohenwallner, N. Troppmair, L. Panzenboeck, C. Kasper, Y. El Abiead, G. Koellensperger, L. M. Lamp, J. Hartler, D. Egger, E. Rampler, JACS Au 2022, 2, 2466-2480.
[3] [3] J. Hartler, A. M. Armando, M. Trötzmüller, E. A. Dennis, H. C. Köfeler, O. Quehenberger, Anal. Chem. 2020, 92, 14054-14062.

Quantitative tracing of fatty acid metabolism in primary hepatocytes

Theja Sajeevan (1), Prof. Dr. phil. nat. Christoph Thiele Thiele (2)

(1) LIMES Institute, University of Bonn, (2) LIMES Institution, University of Bonn

Type of abstract: ORAL

Introduction and Aims:

Hepatocyte lipid metabolism is central to whole-body lipid homeostasis and is affected in numerous metabolic and neurodegenerative disorders. Despite its importance, current understanding of hepatic lipid metabolism is limited in kinetics with species-level resolution.

Material and Methods:
We present a novel multiplex mass spectrometry technique based on alkyne-labelled fatty acids and click-chemistry, enabling high-sensitivity tracing of fatty acid incorporation into lipid species. This also helps us achieve parallel tracing of more than one (odd-, even-chain, and ^{13}C-labeled) alkyne-labelled fatty acid incorporation in a single experiment.

Results:
We use this technology in primary murine hepatocytes to resolve the relative contributions of the Kennedy pathway, Lands cycle, and PEMT pathway to hepatic PC synthesis and remodelling. We see that approximately 20% of the total PCs are synthesised by the PEMT pathway. Lands cycle contributes to the polyunsaturated fatty acid (PUFA) remodelling of the PCs species. Additionally, we also investigated triacylglycerol (TAG) turnover, revealing evidence for TAG cycling in primary murine hepatocytes.

Conclusions:
This offers novel insight into the dynamic remodelling of hepatic lipids and sets the stage for detailed metabolic flux analysis in health and disease models.

References

[1] 1. Thiele, C., K. Wunderling, and P. Leyendecker. 2019. Multiplexed and single cell tracing of lipid metabolism. Nat Methods 7:2004. doi:10.1038/s41592-019-0593-6.

Unleashing the Potential of Lipidomics for Population Health Research

Jessica Medina[1], Nicolas Goss[1], Gonçalo Dos Santos Correia[2], Rebecca Borreggine[1], Tony Teav[1], Zoltan Kutalik[3], Pedro Marques Vidal[4], Hector Gallart-Ayala[1], Julijana Ivanisevic[1]

[1] Metabolomics and Lipidomics and Lipidomics Platform, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland
[2] Institute of Reproductive and Developmental Biology, Department of Metabolism, Digestion and Reproduction, Imperial College London, London, UK; March of Dimes Prematurity Research Centre at Imperial College London, London, UK
[3] Department of Computational Biology, Faculty of Biology and Medicine, University of Lausanne, Lausanne, Switzerland
[4] Department of Medicine, Internal Medicine, Lausanne University Hospital (CHUV) and University of Lausanne, Lausanne, Switzerland

Type of abstract: ORAL

Lipid metabolism and circulatory lipid levels are closely associated with cardiometabolic health. Consequently, MS-based lipidomics, which enables the selective measurement of hundreds to thousands of lipid species, has emerged as a powerful phenotyping tool in epidemiological and clinical intervention studies. However, achieving high-throughput, reproducible measurement of a broad panel of circulatory lipid species in large-scale studies remains a significant challenge.
We applied a recently developed quantitative LC-MS/MS lipidomics approach to a subset of 1,086 fasting plasma samples from apparently healthy participants in the prospective Lausanne population study. This high-coverage, high-throughput hydrophilic interaction liquid chromatography (HILIC)-based methodology enabled robust measurement of 782 circulatory lipid species spanning 22 lipid classes and covering a concentration range of six orders of magnitude. This was achieved through semi-automated sample preparation using a stable isotope dilution approach and the alternate analysis of NIST plasma reference material as a quality control.
Importantly, the biological variability of individual lipid species was significantly higher than the batch-to-batch analytical variability. Unsupervised clustering further demonstrated the high individuality and sex specificity of the circulatory lipidome. Lipid signatures from the same individuals clustered together over a 10-year period, demonstrating their capacity to capture chemical individuality. The most prominent sex differences were observed for sphingomyelins and ether-linked phospholipids, which were present at significantly higher concentrations in female plasma. While the origin of these sex differences remains to be determined, they are likely sex hormone-driven, reflecting differential activity of enzymes involved in lipid metabolism (regardless of age).
These findings underscore the importance of establishing stratified reference intervals based on age, sex, and hormonal status. They also highlight the need to account for sex differences in cardiometabolic risk assessment, clinical diagnostics, and personalized treatment strategies.

References

DOI: 10.1021/acs.analchem.2c02598
DOI: 10.1016/j.jlr.2025.100780

Lipidomic Profiling of SARS-CoV-2-Infected Mice: Viral Dose-Dependent and Organ-Specific Alterations

Myeong Hee Moon (1)

(1) Yonsei University

Type of abstract: ORAL

SARS-CoV-2 infection induces profound metabolic disruptions, yet its impact on lipid homeostasis across organs remains unclear. We performed comprehensive lipidomic profiling of brain, spleen, lung, liver, and serum from K18-hACE2 mice infected with low-dose (LD, 1×10^2 PFU/mL) or high-dose (HD, 1×10^5 PFU/mL) SARS-CoV-2 by nUHPLC-ESI-MS/MS. Principal component analysis showed that LD-infected mice largely recovered lipid profiles by 10-14 days post-infection (dpi), while HD-infected mice exhibited persistent lipidomic disruptions correlating with mortality. Volcano plots revealed early significant reductions in key lipids at 1-2 dpi across tissues, with partial recovery in LD but sustained dysregulation in HD. Notably, phosphatidylcholine (PC) and phosphatidylethanolamine (PE) showed marked declines and incomplete recovery in HD groups, reflected by persistent PC/PE ratio alterations indicating membrane instability. Phosphatidylinositol (PI) and its major species PI 38:4 rose sharply in HD serum, suggesting a role in immune dysregulation and inflammation during severe infection. Cholesteryl esters (CE) increased in brain and spleen under HD infection, suggesting central nervous system and immune dysfunction. Unsaturated lysophospholipids (LPC, LPI) decreased significantly in HD mice, indicating inflammatory membrane remodeling. Collectively, these results demonstrate dose- and tissue-dependent lipidomic alterations during SARS-CoV-2 infection, reveal potential mechanisms of organ damage involving disrupted lipid metabolism and signaling, and suggest candidate lipid biomarkers for assessing infection severity and recovery. Our findings underscore the importance of lipidomic profiling in understanding COVID-19 pathogenesis and monitoring disease progression.

Characterization of lipid biomass derived from (precision) fermentation through high-resolution mass spectrometry

Tavazzi Isabelle (1), Francesca Giuffrida (2), Dionisi Fabiola (3), André Axel (1)

(1) Nestlé Research, (2) Societes des Produits Nestlé, (3) Nestlé research

Type of abstract: ORAL

Lipidomic Analysis Reveals Suaeda maritima as a Sustainable Source of Food Lipids through Aquaponic Cultivation

Rosário Domingues (1), Pinho Marisa, Cremades Javier

(1) University of Aveiro

Type of abstract: ORAL

FAMetA: a mass-isototopogue-based tool for the comprehensive analysis of fatty acid metabolism

Juan Carlos Garcia Cañaveras (1), Maria Isabel Alcoriza-Balaguer (1), Marta LLatas (1), Marta Roca (1), Marta Benet (1), Agustin Lahoz (1)

(1) IIS-La Fe

Type of abstract: ORAL

Introduction and aims:

Dysregulated fatty acid (FA) metabolism is a common feature in pathologies as obesity, NAFLD or cancer. The use of stable isotope tracers and MS is the gold standard method for the analysis of FA metabolism. Yet current state-of-the-art tools provide limited information about FA biosynthetic routes. Thus, our aim is to develop a bioinformatics tool capable of providing a comprehensive assessment of fatty acid metabolism based on ^{13}C mass-isotopologue profiles incorporated into newly synthesized fatty acids *in vivo* and *in vitro*.

Materials and Methods:

The non-small cell lung cancer (NSCLC) cell line A549 and primary cultured activated $CD8^+$ T cells were used. Cells were incubated with U-^{13}C tracers for 72h in complete RPMI media. Cellular lipids were extracted and saponified to enable the analysis of total FA acids. FAs were analyzed by an in-house developed RP-LC-MS method that enables the separation and detection of the most biologically relevant FA species[1]. Data pre-processing was performed with LipidMS *(2)*. All the functions and algorithms required for the analysis of FA metabolism were implemented in R. The package FAMetA is available via CRAN (https://CRAN.R-project.org/package=FAMetA)[2]

Results:

We have developed FAMetA, an R-package and a web-based application (www.fameta.es) that uses ^{13}C mass-isotopologue profiles to estimate not only FA import, synthesis and elongation, but also desaturation. For 14 and 16-carbon FAs, de novo lipogenesis parameters [import (I), de novo lipogenesis/synthesis of FA(16:0) (S), and the contribution of the labeled nutrient to the m+0, m+1 and m+2 acetyl-coA pool (D0, D1, D2, respectively)] are estimated. For 18 to 26-carbons FAs, up to five elongation terms (En) are included. FAMetA workflow covers all the functionalities needed for MS data analysis and graphical representation. Compared to previous tools, FAMetA offers better mass isotopologue data fitting thanks to the use of quasi-multinomial distribution; better assessment of FA elongation due to the direct estimation of each step within a specific FA synthesis pathway; and systematic estimation of desaturations (Δ) based on the synthesis parameters of the precursor (S or E) and product FAs (S' or E') where Δ = S'/S or E'/E. The information provided by FAMetA allows to decipher both patterns of global changes and detailed information on alterations in the synthesis route of FAs of interest as demonstrated using a variety of in vitro and in vivo applications. The combination of U-^{13}C-glucose and inhibitors of FASN, SCD1 and FADS2 incubations with FAMetA data analysis enabled the description of the FA biosynthesis network in the NSCLC cell line A549, and the discovery of 12 novel FAs through the metabolic reconstruction of their synthesis route.

Conclusions:

We present FAMetA, an R-package and a web-based application that enables the characterization of FA metabolism from U-^{13}C incubations with unprecedented resolution. The comprehensive characterization of FA biosynthesis and the easy-to-interpret graphical representations offered by FAMetA may provide new insights into the role of FAs in cell biology and in the pathogenesis of many diseases.

References

[1] Alcoriza-Balaguer MI, et al. (2023) Briefings in Bioinformatics, 4: bbad064. PMID: 36857618
[2] Alcoriza-Balaguer MI, et al. (2022) Bioinformatics, 38: 4826-4828. PMID: 36005855

Human Milk Extracellular Vesicles: a lipid shuttle to the preterm infant's gut?

Julia Kuligowski (1), Jose Luis Moreno-Casillas (1), Laura Ripoll (1), Emmanuel Rumba Matano (1), Isabel Ten-Doménech (2), Marta Gómez-Ferrer (1), María Gormaz (1), Anna Parra-Llorca (3), Pilar Sepúlveda (1), Danuta Dudzik (4), Guillermo Quintás (5)

(1) Health Research Institute La Fe (IIS La Fe), (2) University of Valencia, (3) University & Polytechnic Hospital La Fe (HULAFE), (4) Medical University of Gdańsk, (5) Leitat Technological Center

Type of abstract: ORAL

Introduction and Aims: Human milk (HM) has demonstrated protective effects against necrotizing enterocolitis (NEC), a severe gastrointestinal disorder that primarily affects premature neonates. HM extracellular vesicles (HMEVs) are nanoparticles secreted by cells sheathed in a lipid bilayer that are believed to play a critical role in mitigating NEC risk, though exact mechanisms remain to be elucidated. Notably, HMEVs show resistance to digestion, allowing their bioactive contents to reach the infant's intestinal tract intact, underscoring their potential to reduce NEC. The aim is to study the lipidome of HMEVs using complementary analytical techniques and to test their potential functionality in a 3D in-vitro gut model.

Material and Methods: HM samples were collected using sterilized breast milk pumps (N=50). Additionally, HM samples from mothers of preterm infants that developed NEC and a control group (no NEC) were recruited (N=20). EVs were isolated using Size Exclusion Chromatography (SEC) followed by one-step ultracentrifugation. Quality control included Attenuated Total Reflectance-Fourier Transform Infrared (ATR-FTIR) spectroscopy (total protein, total fat), Tunable Resistive Pulse Sensing (TRPS, particle count and concentration), Transmission Electron Microscopy (TEM, size and shape), Western Blot (membrane proteins), and ExoView® analysis (membrane protein co-localization). Oxylipins were analyzed using a targeted/semi-targeted method via Ultra Performance Liquid Chromatography-tandem Mass Spectrometry (UPLC-MS/MS)[1]. Fatty Acid Methyl Esters (FAMES) were profiled by GC-MS after basic hydrolysis and derivatization. An untargeted LC-qTOFMS lipidomic analysis was also performed[2]. In-vitro assays were conducted using the MIMETAS® platform to study the effects of EVs on tight junctions (immunohistochemical staining), inflammation (cytokines), and cytotoxicity (LDH).

Results: Vesicle identity and purity were confirmed using TEM, Western Blot, and ExoView®. EV concentration was $7.3\times10^{11} \pm 9.4\times10^{10}$ particles/mL (mode = 4.2×10^{11}), with a mode size of 106 nm. Purity reached $(2.1 \pm 0.2)\times10^{14}$ particles/g protein (mode = 3.5×10^{14}). Targeted oxylipin profiling of ten fresh milk samples quantified seven oxylipins, including 12,13-DiHOME, 9,10-DiHOME, 14-HDHA, and several EETs (11,12-EET, 8,9-EET, 14,15-EET). These bioactive lipids are implicated in inflammatory regulation, suggesting a potential immune-modulating role for HMEVs. Thirty-two FAMES were detected at concentrations from 1.3×10^{-9} to 2.0×10^{-6} mol/L. Comparative analysis showed HM was relatively enriched in oleic and linoleic acid, while HMEVs had higher levels of arachidonic acid (AA), docosanoate, eicosapentaenoic acid, and docosahexaenoic acid (DHA). The AA/DHA ratio in HMEVs was 1.9, considered beneficial for neurological and visual development. Untargeted lipidomics annotated 539 features in positive and 132 in negative electrospray ionization modes. The most frequently detected classes were triacylglycerols (29%), glycerophospholipids (16%), and phosphosphingolipids (11%). Univariate and multivariate analyses showed no significant lipidomic differences between HMEVs from mothers of infants with and without NEC. This suggests NEC susceptibility may be more influenced by infant-related factors such as gestational age, immune status, or gut maturity than by HMEV lipid composition. In a Caco-2 in-vitro 3D gut epithelial model, HMEVs showed no cytotoxicity compared to controls after single (48 h) or repeated (96 h) treatments. HMEV administration enhanced epithelial barrier integrity, demonstrated by increased E-cadherin expression after one and three treatments, and upregulation of occludin-1 after three. HMEV exposure also reduced TNF-α levels, while IL-6 remained unchanged, indicating a selective anti-inflammatory effect.

Conclusions: This study provides a detailed lipidomic characterization and functional assessment of HMEVs, confirming their potential role in protecting the developing gut of preterm infants. Future research will explore their application as a dietary supplement aimed at preventing NEC.

References

[1] Albiach-Delgado A., et al. (2025) Analytica Chimica Acta, 1313:342759. PMID: 38862207
[2] Ten-Doménech I., et al. (2024) Chemometrics and Intelligent Laboratory Systems, 246:105070.

Dynamic Remodeling of Anti-Inflammatory Palmitoleic Acid and Its Isomers in Activated Monocytes and Macrophages

Miguel Ángel Bermúdez (1), Alma María Astudillo (2), Clara Meana (2), Alfonso Pérez-Encabo (3), Álvaro Garrido (2), Laura Pereira (2), Teresa Garrido (2), María de los Ángeles Balboa (2), Jesús Balsinde (2)

(1) Universidad Católica de Ávila / Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Valladolid, (2) Instituto de Biología y Genética Molecular, Consejo Superior de Investigaciones Científicas (CSIC), Universidad de Valladolid, (3) Instituto CINQUIMA, Departamento de Química Orgánica, Universidad de Valladolid

Type of abstract: ORAL

Introduction and Aims: Monounsaturated fatty acids (MUFAs), particularly palmitoleic acid (16:1n-7) and its positional isomers (hypogeic acid, 16:1n-9; sapienic acid, 16:1n-10), are recognized for their anti-inflammatory properties and potential roles as biomarkers in metabolic diseases. Despite their biological significance, the precise mechanisms governing their cellular levels, distribution, and mobilization within lipid pools during immune cell activation remain largely unexplored. This research aimed to characterize the endogenous distribution and dynamic remodeling of 16:1 fatty acids in both human monocytes and mouse peritoneal macrophages, investigate their release upon various inflammatory stimulations, identify the specific phospholipase A2 enzymes involved in their mobilization, and determine if these released fatty acids are converted into other bioactive lipid mediators.

Material and Methods: Human peripheral blood monocytes and resident mouse peritoneal macrophages were cultured and stimulated with diverse inflammatory agonists, including opsonized zymosan, mannan plus laminarin, calcium ionophore A23187, and bacterial lipopolysaccharide (LPS). The distribution and levels of 16:1 fatty acids and their molecular species within phospholipid classes were quantitatively analyzed using GC/MS and LC/MS. To elucidate enzymatic involvement, pharmacological inhibitors targeting group IVA cytosolic phospholipase A2 (cPLA2α), group VIA calcium-independent phospholipase A2 (iPLA2-VIA), and CoA-independent transacylase (CoA-IT) were employed. Additionally, an iPLA2-VIA antisense oligonucleotide was used to confirm enzyme specificity. Deuterated 16:1n-7 and 16:1n-9 were utilized as tracers to monitor the remodeling of these fatty acids into other lipid species.

Results: In resting human monocytes and mouse macrophages, over 90% of total cellular 16:1 fatty acids were concentrated in phosphatidylcholine (PC), predominantly in the PC(16:0/16:1) species, showing minimal inter-phospholipid movement. However, upon stimulation with inflammatory agents, a significant time-dependent decrease in 16:1-containing PC species was consistently observed in both cell types, paralleled by an increase in free 16:1 fatty acids. This release was not isomer-specific. Pharmacological and antisense inhibition studies conclusively demonstrated that iPLA2-VIA is the key enzyme mediating 16:1 fatty acid mobilization from PC in both human and mouse cells, while cPLA2α and CoA-IT showed no discernible effect on this specific release.

Furthermore, a small but significant portion of the released 16:1 fatty acids was rapidly transferred to phosphatidylinositol (PI) in human monocytes. In mouse macrophages, this remodeling also led to the enrichment of PI and the formation of 16:1-containing FAHFA, which increased upon activation. The transfer to PI was found to be a CoA-dependent process, with cPLA2 α playing a role in generating the necessary lysoPI acceptors in human monocytes. The multiplicity of effects for palmitoleic acid and its isomers suggests overlapping actions of multiple isomers due to compartmentalization within the cell.

Conclusions: This research establishes a novel CoA-dependent pathway for the dynamic remodeling and utilization of 16:1 fatty acids in activated human monocytes and mouse peritoneal macrophages. The process involves the participation of two distinct intracellular phospholipase A2 enzymes: iPLA2-VIA is identified as the primary enzyme responsible for releasing free 16:1 from PC, while cPLA2α contributes by generating lysophospholipid acceptors. The released 16:1 fatty acids are not only increased in their free form but are also actively transferred to enrich specific PI molecular species and to form bioactive FAHFA. These findings suggest that the accumulation of 16:1 in defined lipid species during immune cell activation may underlie some of the anti-inflammatory and other biological effects attributed to palmitoleic acid and its positional isomers, providing new insights into lipid signaling during innate immune responses.

References

[1] Bermúdez MA, et al. (2024) Biomolecules, 14:707. PMID: 38884638.
[2] Astudillo AM, et al. (2020) Biomedicines, 9:480. PMID: 33167733

ECOLIPID DH5: a highly curated lipidome characterization of Escherichia coli DH5α strain generated by multiplatform deep lipidomic profiling

Miguel Fernández-García (1), Henrique Santos-Oliveira (2), Lida Garzón-Gordo (2), Antonia García (2)

(1) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain; Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain, (2) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Madrid, Spain

Type of abstract: ORAL

Introduction and aims:

Lipidome databases comprehensively characterize the lipidome of a biological matrix, facilitating global lipidomic analyses. However, detailed lipidome characterizations of bacterial model organisms with targeted validation remain scarce, limiting the translation from global lipidomics to a deeper systems-level understanding of membrane lipid biochemistry. To address this methodological gap, we aimed to provide a highly-curated database of *Escherichia coli* with enhanced coverage and structural detail.

Materials and methods:

We used *E. coli* DH5α, a strain widely used in molecular cloning and genetic engineering. We analyzed concentrated bacterial lipid extracts by a previously-optimized reversed-phase liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (RPLC-ESI(+/-)-QTOF/MS) method[1] using an Agilent 1290 II LC system coupled to an Agilent 6545 Q-TOF mass spectrometer. Lipid annotations were validated for phosphatidylethanolamines (PE) and phosphatidylglycerols (PG) by targeted LC-ESI(+/-)-QqQ/MS, using an Agilent 1290 II LC system coupled to an Agilent 6460 QqQ mass spectrometer. Lipid annotation combined software assisted MS/MS annotation, literature-driven in silico prediction of lipid molecular species, and comprehensive in silico MS/MS assessment of coelutions, all followed by MS and MS/MS manual inspection according to reference criteria[1]. All annotations were subjected to manual curation and quality assessment procedures involving inter-lipid subclass and intra-lipid subclass consistency with a reference set of annotations and standards in multiple pieces of information, as previously performed for NIST SRM 1950 human plasma reference material[1].

Results:

Our approach culminated in ECOLIPID DH5, a highly curated database with >350 lipid molecular species, representing a >50% increase in coverage compared to the *E. coli* reference lipidome characterization[2]. Our database provides the most curated lipidome characterization of conserved bacterial lipid metabolism up to date including 98 phosphatidylethanolamines, 68 phosphatidylglycerols and >50 cardiolipin molecular species within a 19-min runtime.

Conclusions:

To the best of our knowledge, our database represents the most comprehensive and detailed *E. coli* lipidome characterization to date. ECOLIPID DH5 streamlines lipidomics data processing and curation, it is easily scalable to other *E. coli* strains and supports applications in multiple fields including lipid biochemistry, microbial genetics, synthetic biology, lipidome engineering and infectious disease research.

References

[1] Martínez, S, et al. (2024) Journal of Lipid Research, 65(11):100671. PMID: 39395790
[2] Jeucken, A et al. (2019) Cell Reports, 27(5):1597-1606. PMID: 31042483

Cerebrotendinous Xanthomatosis: 13-Year Follow-Up of LDL-Apheresis Therapy with Plasma 7α-Hydroxy-4-cholesten-3-one Monitoring

Silke Matysik (1), Brosig Andreas (1)

(1) University Hospital Regensburg

Type of abstract: ORAL

Cerebrotendinous xanthomatosis (CTX) is a rare, inherited, autosomal recessive lipid-storage disorder caused by sterol 27-hydroxylase deficiency. The sterol 27-hydroxylase enzyme, encoded by CYP27A1, is responsible for degrading the cholesterol side chain to produce bile acids. As a consequence of enzyme dysfunction, a marked bile acid deficiency occurs, accompanied by the accumulation of bile acid precursors in plasma and tissues. In CTX, particularly elevated cholestanol concentrations and increased excretion of bile alcohol glucuronides are observed, both of which can be used as biochemical markers for diagnosis. Cholestanol formation is thought to be the primary driver of xanthoma development. In vivo, cholestanol is derived from 7α-hydroxylated cholesterol metabolites, among which 7α-hydroxy-4-cholesten-3-one is presumably the most important, showing up to a 100-fold increase in the plasma of affected individuals.

We report the case of a 42-year-old man with CTX who has been treated with chenodeoxycholic acid, simvastatin, and low-density lipoprotein (LDL) apheresis. As the majority of toxic compounds (e.g., cholestanol) accumulate within LDL particles, weekly therapeutic LDL apheresis may improve the clinical course of CTX by reducing toxic lipids and slowing disease progression.

LDL apheresis has been performed weekly for the past 13 years. During the first six months, treatment efficacy was monitored by quantitative determination of plasma 7α-hydroxy-4-cholesten-3-one using LC-MS/MS. Each LDL apheresis session effectively cleared 7α-hydroxy-4-cholesten-3-one from plasma, but levels returned to baseline within seven days (mean pre-apheresis concentration: 241 ng/mL).

Here, we present a 13-year follow-up on the long-term effectiveness of LDL apheresis. The patient remained highly adherent to therapy and has shown no significant clinical deterioration over this period. LDL, HDL, and total cholesterol levels were monitored before and after every treatment. Additionally, over a three-month period, 7α-hydroxy-4-cholesten-3-one was remeasured to reassess hepatic synthesis and confirm the continued necessity of weekly apheresis. Pre-apheresis levels were comparable to those measured 13 years earlier (mean 236 ng/mL) but exhibited markedly less fluctuation. These findings support the sustained need for effective lipid-lowering therapy in CTX.

Screening Method for Pancreatic Cancer Using Lipidomic Profiling of Plasma or Serum: A Pilot Study

Ondřej Peterka (1), Robert Jirásko (1), Zuzana Dolečková (2), Mária Dosoudilová (2), Michal Holčapek (1)

(1) University of Pardubice, (2) Lipidica, a.s.

Type of abstract: ORAL

Introduction and Aims:

Pancreatic cancer is among the deadliest malignancies, with a 5-year relative survival rate of only 13% because fewer than 20% of patients are diagnosed at a resectable stage due to asymptomatic or vague symptoms, leading to detection at late-stage. The carbohydrate antigen (CA) 19-9 is the only biomarker routinely used in pancreatic ductal adenocarcinoma (PDAC) diagnostics, but it has low sensitivity for early-stage disease and lacks specificity, limiting its utility in screening programs. Imaging modalities, such as endoscopic ultrasonography (EUS), magnetic resonance imaging (MRI), and computed tomography (CT), are the standard diagnostic tools for early diagnosis of PDAC, but these methods are time-consuming, technically demanding, and often uncomfortable or invasive for patients. In the general population, the lifetime risk of developing PDAC is approximately 1.6%, but the high-risk individuals (HRI) with genetic susceptibility syndromes, a familial pancreatic cancer, or hereditary pancreatitis exceed 5% predetermining HRI for the screening program. A noninvasive, high-throughput method capable of detecting reliable biomarkers in body fluids could be a breakthrough in the early diagnosis of PDAC.

Material and Methods:

The modified Folch extraction procedure was used for the extraction of lipids from 25 µL of EDTA human plasma or serum spiked with 20 µL of IS-Mix before extraction. The ultrahigh-performance supercritical fluid chromatography was connected to a Xevo G2 XS QTOF mass spectrometer for high-resolution MS data acquisition. Gradient elution with total run time 8.0 min was set. LipidQuant 2.1 [1] was used for the calculation of lipid concentrations in biological samples.

Results:

In Phase I, we investigated the effects of gender and sample matrix on lipidomic profiling and model performance for defined lipid classes. Plasma and serum matrices showed no substantial differences in lipid profiles, but slightly better performance and predictive ability were provided for plasma models. On the contrary, significant differences were observed between the lipidomic profiles of healthy controls (n = 218) and patients with PDAC (n = 177) for both matrices. The downregulation is observed mainly in ceramides and sphingomyelins with very long N-acyl chains and in plasmenyl/ether phospholipids compared to upregulated diacylglycerols.

In Phase II, we applied the final methodology to HRI with the aim of evaluating its suitability for screening purposes. In total, 93 HRI samples were prospectively collected in collaboration with two national pilot studies in the Czech Republic and the diagnostic performance of the lipidomic profiling method was compared with conventional imaging methods, tumor biomarkers CA 19-9, and carcinoembryonic antigen.

Conclusions:

We present the robust methodology optimized for the clinical application and the same dysregulated lipid patterns were confirmed in prospectively collected samples, with 54% of early stages (T1 and T2). Across all stages, the average sensitivity, specificity, and accuracy were 98%, 99%, and 99% in training, and 92%, 100%, and 96% in validation sets, which is an improvement of 4-10% compared to our previous method [2] and approximately 30% higher sensitivity than CA 19-9. The methodology also demonstrated 96% specificity for HRI when benchmarked against results from EUS and MRI. Based on these findings, a multicenter clinical trial has been initiated (registered at ClinicalTrials.gov, NCT04241796) in collaboration

with 14 clinical centers in the Czech Republic.

This work was supported by the ERC Adv grant No. 101095860.

References

[1] Chocholoušková M, et al (2024) Chemometr. Intell. Lab. Syst., 251:105169.
[2] Wolrab D, et al (2022) Nature Communications, 13:124. PMID: 8748654

Quantitative Analysis of Monohydroxy Sterols and Vitamin E in Human Plasma Combining Derivatization and RP-UHPLC/MS/MS in Negative Ion Mode

Yasmin Kadyrbekova (1), Ondrej Peterka (1), Robert Jirasko (1), Michal Holcapek (1)

(1) University of Pardubice

Type of abstract: ORAL

Introduction and Aims:

Cholesterol plays a crucial role in mammals and it is a major precursor to other biomolecules like bile acids, oxysterols, and steroid hormones. Moreover, dysregulation of its biosynthesis contributes to cardiovascular diseases, Alzheimer's disease, and cancer. The determination of sterol concentrations can help to understand metabolic alterations and pathogenesis of serious diseases.

Mass spectrometry (MS) coupled with chromatography is the key approach for sterol identification and quantification. Although gas chromatography was once dominant, liquid chromatography (LC) has become the most preferred method. Due to low ionization efficiency and high in-source fragmentation, sterols often produce intense fragment ions while the molecular ion is weak or absent, resulting in low sensitivity for its detection. However, this problem can be solved by the derivatization of free hydroxy group.

We have previously optimized derivatization method using 3-(chlorosulfonyl)benzoic acid (Cl-SBA), enabling detection of acylglycerols, free sterols, and prenols by reversed-phase (RP) LC and multiple reaction monitoring (MRM) transitions in negative - ion mode on a QqQ instrument. This approach combines RP-UHPLC/MS/MS and the use of specific fragmentation patterns, significantly reduces limits of detection, reaching 15 – 25 pmol/mL for free sterols in plasma.[1]

In this study, we focused on the quantitation of monohydroxy sterols and vitamin E, enabling significant reduction of analysis time.

Material and Methods:

The pooled plasma sample used for the validation of the method and identification was prepared by mixing aliquots of 140 human plasma samples from healthy individuals.

Lipids were extracted from 25 µL of human plasma spiked with 20 µL of IS-Mix using modified Folch extraction. The extracts were evaporated and, subsequently, derivatized with Cl-SBA in diluted pyridine[1], and re-extracted using Folch to eliminate unwanted products from the reaction. The final derivatives were analyzed using a 10-minute RP-UHPLC method on a QqQ mass spectrometer. The MS conditions were optimized in negative-ion MRM mode using derivatized standards

Results:

To optimize sample preparation for targeted lipids, five different extraction protocols including one- and two-phases approaches were compared. The modified Folch extraction provided the highest yield (>90% recovery) and excellent repeatability (RSD <9%, n=10). Using the newly optimized conditions, we have successfully increased the number of identified free monohydroxy sterols from 14 to 30. The newly optimized method was then validated following recommendations for bioanalytical methods[2] using human plasma spiked by IS-Mix. The validated method was then applied to the quantitative analysis of free monohydroxy sterols in human plasma.

Conclusion:

We developed a rapid, validated RP-UHPLC/MS/MS method for quantification of free monohydroxy sterols and vitamin E in human plasma. We increased the number of identified sterol species, identifying 30 monohydroxy sterols based on a combination of MRM transitions, retention dependencies, and derivatization tags. Moreover, most of the identified lipid species were confirmed by authentic standards. This method provides sensitive, high-throughput free monohydroxy sterol profiling suitable for clinical and biochemical research.

This work was supported by European Research Council (ERC) projects No. 101095860.

References:

[1] Peterka O, et al. (2025) Anal. Chem., 97: 7157-7164. DOI: 10.1021/acs.analchem.4c06496.

[2] Wolrab D, et al. (2020) Analytical and Bioanalytical Chemistry, 412: 2375-2388. DOI: 10.1007/s00216-020-02473-3.

Brain-Region-Specific Lipid Dysregulation in L-DOPA-Induced Dyskinesia in a Primate Model of Parkinson's Disease

Ibrahim Kaya (1), Theodosia Vallianatou (1), Anna Nilsson (1), Patrik Bjärterot (1), Reza Shariatgorji (1), Per Svenningsson (2), Erwan Bezard (3), Per E. Andrén (1)

(1) Uppsala University, (2) Karolinska Institute, (3) University of Bordeaux

Type of abstract: ORAL

Synthesis and Biological Evaluation of a New Dihydroceramide Desaturase Inhibitor for the Treatment of MASLD

Karla Ceballos (1), Jose Luis Abad (2), Bohdan Babiy (1), Álvaro Hidalgo (1), Yanara Ferrer (1), Silvia Sacristán (3), Rebeca Busto (3), Javier Martínez-Botas (3), Jordi Bujons (2), Cristina Bello (2), Diego Abad-Montero (2), Gemma Fábrias (2), Óscar Pastor (1)

(1) Servicio de Bioquímica- Clínica, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain, (2) Instituto de Química Avanzada de Cataluña-CSIC, (3) Servicio de Bioquímica- Investigación, Hospital Universitario Ramón y Cajal, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain

Type of abstract: ORAL

ω-hydroxylation - a yet undescribed novel hepatic pathway for the detoxification of 1-deoxysphingolipids

Thorsten Hornemann (1), Majcher Adam (2)

(1) Universtiy Zurich, (2) University Zurich

Type of abstract: ORAL

Enhancing Lipid Annotation Confidence in NIST® SRM® 1950 with C=C Double Bond Position Determination via EIEIO-MS

Sara Martínez López (1), Hana Cermakova (1), Miguel Fernández-García (1), Sara Londoño-Osorio (1), Ana Gradillas (1), Michael Witting (2), Coral Barbas (1)

(1) Centro de Metabolómica y Bioanálisis (CEMBIO), (2) Metabolomics and Proteomics Core Facility, Helmholtz Zentrum München

Type of abstract: ORAL

Instrument QC for shotgun lipidomics

Silvia Radrezza (1), HongKee Moon (2), Lena Herseman (2), Andrej Schevchenko (1)

(1) Max Planck Institute of Molecular Cell Biology and Genetics, (2) SciComp Facility, Max Planck Institute of Molecular Cell Biology and Genetics

Type of abstract: ORAL

Standardisation in analytical chemistry is crucial for improving data quality, reproducibility, and comparability across studies and laboratories. In lipidomics, inconsistent methods can translate into data discrepancies, making it difficult to interpret results and clinical translation. While there has been increasing attention and efforts on harmonising sample preparation and data analyses [1], instrument performance remains under-addressed. Indeed, to our knowledge, there are no consistent metrics to evaluate, monitor, or troubleshoot mass spectrometer performance – explaining quantification inconsistencies often observed in inter-lab studies. Although shotgun mass spectrometry is a stable and reliable method for the direct infusion of the sample at a constant flow, this stability should not be taken for granted. Uncalibrated instruments, suboptimal settings, or contamination can significantly alter the results. To quickly and reliably verify that we are operating under optimal conditions, we developed a simple and fast workflow for shotgun mass spectrometry lipidomics. In detail, a commercialised standard mix (Ultimate SplashMix) is diluted, plated and analysed in a 3-minute run. The resulting mass list is then input into an in-house-developed and open-access “QC Shotgun” software to check and track over time seven key parameters for shotgun lipidomics analyses (mass accuracy, mass resolution, isotopic distribution, lipid class response, ion source fragmentation, sensitivity and fragmentation efficiency). Our evaluation study involved 10 labs operating shotgun mass spectrometry to collect representative and diverse data under several daily conditions (e.g., uncalibrated instrument, different solvent salt, etc.). Over four months, the combined data provided valuable insights into the method's stability, the software's ability to identify anomalies, and the sources of minor variations across the contributing labs. These findings, along with the positive and interested feedback from the contributing labs, underscore the importance of implementing quality control checks for instrumentation, complemented by standardised sample handling.

References

[1] Lipidomics Standards Initiative Consortium. Lipidomics needs more standardization. Nat Metab 1, 745-747 (2019)

A Mammalian Tissue Standard Reference Material for Improved Lipidomic and Metabolomic Interoperability

Justine BERTRAND-MICHEL (1), Soullier Julia, Monteiro Jeremy, Perrion Theo, Boissin Louise, Lefevre Antoine, Traikal Mounir, Joly Charlotte, MArtineau Estelle, MArchand Jeremy, canlet cecile, jamin Emilien, Guitton Yann, Nadal Lydia, Colsh Benoit, Castelli Florence, Durand Stéphanie, Pujos-Guillot Estelle

(1) MetaboHUB_MetaToul

Type of abstract: ORAL

A Mammalian Tissue Standard Reference Material for Improved Lipidomic and Metabolomic Interoperability

Justine Bertrand-Michel1,2#*, Julia Soulier1,2, Jérémy Monteiro1,3, Théo Perion1,4, Louise Boissin1,5, Antoine Lefevre1,3,, Mounir Traika 1,6, Charlotte Joly1,6, Estelle Martineau1,7, Jérémy Marchand1,7, Cécile Canlet1,4, Emilien L. Jamin1,4, Yann Guitton1,5 , Lydia Nadal1,3, Benoit Colsh1,8, Florence Castelli1,8, Stéphanie Durand1,6, Estelle Pujos-Guillot1,6
1MetaboHUB, Toulouse, France
2I2MC Lipidomic, Inserm,Toulouse, France

3Plateforme de Métabolomique et d'Analyses Chimiques, US61 ASB, Université de Tours, CHRU Tours, Inserm, Tours, France

4Toxalim, Université de Toulouse, INRAE, ENVT, EI-Purpan, Toulouse, France

5Oniris, INRAE Oniris, LABERCA, Nantes, France Department, Institution, City, Country

6Université Clermont Auvergne, INRAE, UNH, Plateforme d'Exploration du Métabolisme,MetaboHUB Clermont, Clermont-Ferrand, France

7 Nantes Université, CNRS, CEISAM, UMR 6230, Nantes, France

8 Université Paris-Saclay, CEA, INRAE, Département Médicaments et Technologies pour la Santé, Gif-sur-Yvette, France

#*justine.bertrand-michel@inserm.fr

Introduction: Lipids are a highly diverse class of biomolecules essential for energy storage, membrane structure, and cellular signalling. Their dysregulation is implicated in numerous diseases and pathological states. Recent technological advances have made lipidomic analysis more accessible, enabling its application across a wide range of samples and study designs. However, like metabolomics, lipidomics relies heavily on mass spectrometry, which–due to variations in instrumentation, operating conditions, and scanning modes–inherently lacks reproducibility. This variability complicates cross-laboratory and longitudinal data comparison thus limiting impact of studies.

Aims: To address these challenges, reference materials (RMs) are systematically analyzed to provide a common benchmark for data comparison. Although frozen human plasma reference materials (e.g., NIST SRM 1950) are available for plasma analyses, and a liver extract (NIST candidate RM 8462) is currently under evaluation, no such standards yet exist for mammalian or plant tissues (1). This project aims to develop a novel RM for mammalian tissues to support both lipidomic and metabolomic analyses. The RM will enhance data robustness and reproducibility, facilitate instrument validation and method development, and improve metabolite annotation.

Materials and Methods: Twelve rats were euthanized, and seven organs (liver, lung, muscle, brain, cerebellum, heart, and intestine) were collected. Tissues were freeze-dried, ground, and homogenized. Partners within the MetaboHub consortium analyzed the lipid and polar metabolite profiles of individual organs as well as a composite extract, using a range of techniques, including LC-MS, GC-MS, SFC-MS, and NMR.

Results: This presentation will summarize the preliminary targeted and untargeted lipidomic and metabolomic data obtained

for each tissue using different analytical platforms. Additionally, mixtures of lipid extracts from various organs were analyzed to identify the combination offering the greatest lipidome diversity.

Conclusions & Perspectives: The detailed characterization of these extracts is nearly complete, though storage stability remains to be evaluated. The mammalian RM has already been integrated into several projects, and ongoing work focuses on optimizing its use and establishing best practices for implementation.

References

[1] (1) Lippa KA, et al. (2022) Metabolomics, 18(4) :24, PMID: 35397018

Shotgun mass spectrometry-based lipid profiling of early atherosclerosis: The AWHS Study

Alessia Ferrarini (1), Valentín Fuster (1), Estefania Núñez (1), Rafael Barrero-Rodríguez (1), Annalaura Mastrangelo (1), Rocio Tarifa (1), Ricardo Magni (1), Borja Ibáñez (1), Enrique Lara-Pezzi (1), Antonio Fernández-Ortiz (1), Jesus Vázquez (1)

(1) Centro Nacional de Investigaciones Cardiovasculares Carlos III, Madrid 28029, Spain

Type of abstract: ORAL

Introduction and Aims:

In its 2025 report, the American Heart Association, stated that cardiovascular diseases (CVDs) remain the leading cause of death worldwide, with incidence continuing to rise since 1990. Atherosclerosis, the primary driver of CVDs, progresses silently over decades and often manifests only at advanced stages or after cardiovascular events. Regardless the advances in personalized medicine, preventing, detecting and understanding subclinical atherosclerosis (SA) remains a major challenge, as traditional risk factors (RFs) are insufficient to identify early-stage or at-risk individuals[1], and its multicomponent features highlight complex mechanisms that demand clarification.

It is now clear that lipids are not just simple firewood to assure energy insource, but key regulators of a plethora of cardiovascular processes. Therefore, detailed lipidomic profiling may offers a transformative point to gain a deeper understanding of SA, in apparently healthy individuals, and improved early detection and risk assessment.

For these reasons, this study aims to characterize the plasma lipidomic signature associated with SA in healthy individuals and to investigate its relationship with SA indicators such as plaque thickness and coronary artery calcium score (CACS).

Material and Methods:

Plasma lipidomes of heathy volunteer from a case-control study nested within the longitudinal Aragon Workers' Health Study (AWHS) cohort (n=350)[2] were retrieved by shotgun mass spectrometry-based lipidomic analysis. Briefly, plasma samples of middle-aged (40 to 59 years of age) male workers were diluted in acidic condition and spiked with Avanti splash lipidomix standard mixture. Lipids were extracted with MTBE and the organic phase was dried-out, resuspended and diluted prior to direct infusion via nanoflow ESI source (Advion Triversa Nanomate) into a Quadrupole-Orbitrap Hybrid mass spectrometer (QExactive, Thermo Fisher Scientific). Lipids from controls and heathy individuals with early signs of atherosclerosis (atherosclerotic plaque in 3 or more vascular territories and/or CACS ≥ 1) were profiled in positive and negative full scan mode with resolution (R) = 140,000, and MS/MS-spectra were acquired in DDA mode with R = 70,000. Data were processed using LipidXplorer software through molecular query fragmentation language (MQFL).

Results:

Shotgun lipidomic analysis enabled the identification and quantification of 650 and 230 lipids species in positive and negative mode, respectively, belonging to 19 major lipid classes. Glycerophospholipids (GPLs), lysoglycerophospholipids (LysoGLPs), sphingomyelins (SMs) and triacyclglycerols (TAGs) showed significant correlation with plaque thickness and CACS. Furthermore, when comparing controls with SA individuals, significant changes in plasma lipids emerged. Linear regression confirmed that a subset of lipids was strongly associated with SA indicators independently of cardiovascular RF, whereas logistic regression analysis highlighted discriminative lipids in participants with early sign of atherosclerosis, after adjusting for RF.

Conclusions:

The characterization of the plasma lipidomic signatures of 350 healthy individuals revealed several lipid species strongly

associated with plaque thickness, CACS and the presence of SA in multiple territories, independently for common cardiovascular RF. Among them, GPLs, together with TAGs and SMs were the most abundant class of lipids associated to SA in seemingly healthy individuals. These findings open new possibilities for targeting novel molecules in the early onset of atherosclerosis and evaluates their potential as independent biomarkers for early diagnosis and risk assessment.

References

[1] Fernández-Friera, L. et al. Prevalence, Vascular Distribution, and Multiterritorial Extent of Subclinical Atherosclerosis in a Middle-Aged Cohort. Circulation 131, 2104-2113 (2015)
[2] Laclaustra, M. et al. Femoral and Carotid Subclinical Atherosclerosis Association With Risk Factors and Coronary Calcium: The AWHS Study. J. Am. Coll. Cardiol. 67, 1263–1274 (2016).

Oxylipin Profiles in Aggressive versus Suppressed Neuroblastoma: Effects of Highly Unsaturated Fatty Acids in a Mouse Model

Tom Brenna (1)

(1) University of Texas at Austin

Type of abstract: ORAL

Integrated DESI-MS Imaging and SFC-QTOF Analysis for Spatial Lipid Characterization in Drosophila melanogaster Larvae

Pauline LE FAOUDER (1), Hanna Kulyk (1), Pavel Melentev (2), Cyrielle Clément (1), François Leulier (2), Justine Bertrand-Michel (1), Floriant Bellvert (1)

(1) MetaboHUB-MetaToul, National Infrastructure of Métabolomiques & Fluxomics (ANR-11-INBS-0010), 31077 Toulouse, France Institut National des Sciences Appliquées - Toulouse, (2) Institut de Génomique Fonctionnelle de Lyon Ecole Normale Supérieure de Lyon, Université Claude Bernard Lyon 1, Centre National de la Recherche Scientifique, Institut National de Recherche pour

Type of abstract: ORAL

Untargeted Metabolomics and Lipidomics Using Blood Microsamples: Insights from Stability, Exercise, and Dietary Intervention Studies

Dennisse Julieth Avella Estupiñan (1)

(1) Afekta Technologies Ltd.

Type of abstract: ORAL

Background:

Blood microsampling (BµS) is an alternative to conventional venous blood sampling, replacing the need for several milliliters of blood with a few drops, which can be obtained, for example, by finger or heel pricking. BµS has emerged as a promising tool for decentralized metabolomics and lipidomics research and health monitoring in clinical applications. It includes the possibility of minimally invasive and self-collecting sampling, which can be performed without clinical settings or professional phlebotomists, and multiple sample replicates over time. However, questions remain regarding sample stability, comparability with conventional matrices such as plasma and whole blood, and sensitivity to reflect lifestyle; for example, the dynamic metabolic changes in response to physiological stressors such as physical activity or diet.

Objective:
We aimed to evaluate the applicability of blood microsamples for untargeted metabolomics and lipidomics. We integrated findings from three complementary studies assessing (i) sample stability, (ii) responsiveness to acute physical activity, and (iii) sensitivity to dietary changes.

Methods:

The analytical strategy combined the results of Liquid Chromatography – Mass Spectrometry (LC-MS) with both reverse-phase and hydrophilic interaction chromatography (HILIC) coupled to a quadrupole-time-of-flight mass analyzer (QToF) using an electrospray ionization source (ESI) in positive and negative modes. The studies focused on the applicability of the blood microsampling device known as Neoteryx Mitra®, a promising quantitative volumetric absorptive microsampling (VAMS) alternative.

Firstly, we evaluated the stability of metabolic and lipidomic profiles of Mitra® samples during one week at room temperature. Samples were stored at -80 °C at 0-, 1-, 3-, and 7-day post-collection to evaluate stability during short-term storage. This study aimed to understand how the metabolic results of BµS can be affected during transportation from patients to analytical centres.

Secondly, Mitra® and matched plasma samples were collected from 25 volunteers (13 men, 12 women) before, immediately after, and 1 hour after a treadmill exercise across two replicate sessions. We aimed to compare the results between plasma as a conventional reference sample versus Mitra® samples.

Lastly, the same 25 volunteers underwent a dietary intervention with two special isocaloric meal types: a whole-food-rich diet (fruits, vegetables, nuts) and an ultra-processed diet (foods such as soda, chips, ham), among other ingredients. Mitra® samples were collected before and after the special meals at 0.5-, 1-, and 3- hours, to monitor postprandial metabolomic changes. The two meals were given with a week's time difference.

Both interventions included a controlled diet during the two-week interventions, and a random, crossover, and counterbalanced design, considering volunteers' weight and physical capabilities.

Preliminary Findings:

In the stability study, we observed distinct performance across lipid classes and more polar metabolites; some metabolites and lipids demonstrated good short-term stability at room temperature, while others' abundance changed more significantly over time. In the exercise intervention, BµS reflected time-dependent shifts in metabolite profiles, overlapping some of the plasma dynamics, reinforcing their potential for capturing the information of physiological states. In the dietary study, BµS reflected diet-specific signatures, including differences in metabolites associated with phytochemical intake and postprandial lipid metabolism.

Conclusion and Outlook:

Together, these three studies demonstrate the potential of BµS for untargeted metabolomics and lipidomics. Preliminary data suggest that BµS can provide stable, representative, and biologically meaningful metabolic information across diverse contexts. These findings support the utility of microsampling for decentralized studies, personalized monitoring, and large-scale clinical or population-based lipidomics research. Future results will provide deeper insight into the robustness of this approach across metabolite classes and intervention types.

References

[1] https://pubmed.ncbi.nlm.nih.gov/38948320/
[2] https://doi.org/10.26434/chemrxiv-2025-7tbxq
[3] https://doi.org/10.3390/metabo13101038
[4] https://www.neoteryx.com/volumetrically-accurate-micro-sampling-vams-collection-devices

Towards a Next-Generation Comprehensive Lipid Analysis Platform and the Standardization of Lipidomics in Japan

Takeshi BAMBA (1), Kazutaka IKEDA (2), Kohta NAKATANI (3), Hirofumi FUJIMOTO (4), Kenta MATSUMOTO (4), Masaki YAMADA (4), Daisuke SAIGUSA (5), Hiroshi TSUGAWA (6)

(1) Medical Institute of Bioregulation, Kyushu University, (2) Kazusa DNA Research Institute, (3) Center for Research Promotion, School of Medicine, Niigata University, (4) Shimadzu Corporation, (5) Graduate School of Pharmaceutical Sciences, Teikyo University, (6) Graduate School of Engineering, Tokyo University of Agriculture and Technology

Type of abstract: ORAL

Based on the theme “Establishment and Operation of a Lipid Center for Lipidomics,” which was selected as a priority initiative by the Council on Competitiveness-Nippon (COCN), Kyushu University, the Kazusa DNA Research Institute, and Shimadzu Corporation are collaborating to develop a fully automated platform for next-generation comprehensive lipid analysis.

We have selected the following three lipid categories as the targets of our analysis and are currently developing analytical systems for them.

· Zone 1: Major lipids (fatty acids, phospholipids, sphingolipids, acylglycerols, etc.)

· Zone 2: Fatty acid metabolites (oxylipins and their metabolites)

· Zone 3: Cholesterol-related metabolites (sterols, oxysterols, steroids, bile acids, fat-soluble vitamins, etc.)

Efforts are also underway to automate the entire workflow–from sample preparation to analysis–to facilitate large-scale data acquisition.

Furthermore, as part of our efforts to promote lipidomics in Japan, we have launched the Japan Hub of the International Lipidomics Society (ILS) and are collaborating with various academic societies to standardize lipidomics technologies and promote the adoption of standardized lipidomics checklists.

Enabling the spatial visualization of brain endocannobinoidome by MALDI-2 Mass Spectrometry Imaging

Eduardo Maria Sommella (1), Emanuela Salviati (1), Fabiana Piscitelli (2), Pietro Campiglia (1)

(1) University of Salerno, Department of Pharmacy, (2) Institute of Biomolecular Chemistry, National Research Council of Italy, Pozzuoli, Italy

Type of abstract: ORAL

Introduction and Aims:

Endocannabinoids (eCBs) are endogenous lipid messengers that primarily bind cannabinoid receptors CB1/CB2 and, together with the enzymes that regulate their biosynthesis and degradation, define the endocannabinoid system. The eCB signaling system plays a key role in the central nervous system, resulting often altered in most neurological disorders. However, spatial mapping of eCBs remains elusive for Mass Spectrometry Imaging (MSI), due to their low concentration and tissue dependent ion suppression that obscure their spatial visualization. Herein, we address this limitation through the optimization and application of a laser-induced post-ionization (MALDI-2) approach.

Material and Methods:

Spatial analyses of eCBs were performed on coronal mouse brain sections at bregma levels -2.06 mm and -2.92 mm using a timsTOF fleX equipped with MALDI-2. An initial comparison of MALDI and MALDI-2 detection capabilities was made by spotting on-tissue a dilution series of both authentic and deuterated internal standards (I.S.) of eCBs 2-AG, AEA, OEA, and PEA, on Wild Type mouse brain slices. Different ion trasmission parameters as well as matrices were evaluated to enhance eCBs response. The regio-specific distribution of the four eCBs in WT mouse brain sections was analyzed by MALDI and MALDI-2-MSI at a 20 µm pixel size, the comparison of MALDI and MALDI-2 was carried out on two consecutive slices from the same bregma level.

Results:

We demonstrate that MALDI-2 boosts the detection of 2-arachidonylglycerol (2-AG) and N-acylethanolamines (AEA, PEA, OEA, SEA, LEA) with respect to MALDI, allowing eCBs to be visualized in the brain at endogenous concentrations only by MALDI-2-MSI. MALDI-2 provided higher sensitivity, yielding an average LOD/LOQ gain (MALDI/MALDI-2) factor of 21. Different normalization approaches were evaluated, including root-mean-square (RMS), Total Ion Count (TIC) and isotopically labeled internal standards (I.S.), with I.S. normalization showing improved pixel to pixel variation and more uniform distribution in specific brain regions, especially for 2-AG and PEA. Furthermore, semi-quantitative values of eCBs could be obtained by single-point calibration strategy and compared with LC-MS/MS data, showing good correlation among the two methods. High spatial resolution down to 5 µm pixel size was evaluated, resulting in the detection of all eCBs and confirming the MALDI-2's potential even with reduced tissue ablation. As proof of concept, the method was applied to map eCBs in a mouse model of mild traumatic brain injury (mTBI), the APP-SWE mice, highlighting differences in the modulation of eCBS in Cortex, Hippocampus and Hypothalamus, revealing valuable biological insights for neuropharmacology.

Conclusions: The developed MALDI-2 MSI approach overcomes MALDI limitations and unlocks the detailed spatial mapping of eCBs in the brain.

Lipid CAT: An Open-Access, User-friendly Contaminant Annotation Tool for MS-Based Lipidomics Workflows

Carlos R. Canez (1), Bowen Yang (1), Liang Li (1)

(1) University of Alberta

Type of abstract: ORAL

Introduction

An often-overlooked issue in mass spectrometry-based lipidomic experiments is the pervasive presence of contaminants from experimental workflows, which interfere and complicate analysis of analytes. Other omics fields, including metabolomics, proteomics, and protein-protein interaction studies, have recognized this problem and developed dedicated contaminant databases that enable reliable annotation of spurious signals, preventing their misclassification as genuine biomolecules. These resources have become indispensable for ensuring accuracy and comprehensiveness in molecular annotation. However, the lipidomics community still lacks a comprehensive solution for contaminant identification. To address this gap, we are working on an open-access resource that compiles data on contaminants commonly encountered in lipidomic workflows. This platform will allow users to query their data, including tandem MS spectra, to distinguish contaminants from analytes of interest and thereby improving the accuracy and reliability of untargeted lipidomic workflows.

Methods

A systematic evaluation of leachables and contaminants originating from polypropylene microcentrifuge tubes, polypropylene micropipette tips, extraction solvents, borosilicate pipettes and borosilicate centrifugation glassware during biphasic lipid extraction protocols (Folch, MTBE, and BuMe) was conducted using isolated testing, blank extractions, and human serum extractions. Extracts and isolated samples were analyzed via reversed-phase liquid chromatography coupled with electrospray ionization mass spectrometry on a Bruker Q-TOF Maxis II, an Agilent 6546 Q-TOF, and a Thermo Orbitrap Exploris 240.

A Python-based pipeline was developed for database construction, specifically designed to improve MS/MS spectral matching by enhancing clustering performance through the implementation of the Density-Based Spatial Clustering of Applications with Noise (HDBSCAN) algorithm. Building on this foundation, we developed LipidCAT, a user-friendly software application built with Flask and React frameworks.

Results and Discussion

All tested materials introduced distinct arrays of contaminants and varying levels of contamination. Borosilicate glassware has long been the standard material for lipidomic extractions due to its compatibility with organic solvents. Although glassware from certain manufacturers can introduce higher levels of contamination, products from established suppliers such as Corning and Kimble & Chase contribute only a modest set of low-intensity contaminants.[1]

At present, plasticware has increasingly replaced glassware for lipid extraction and sample processing, among both experienced lipidomicists and newcomers, due to its cost-effectiveness and compatibility with automation. However, even polypropylene plasticware marketed as “organic solvent-resistant” leaches substantial mass spectral contamination when used in Folch, MTBE, or BuMe biphasic extractions. The extent of this contamination varies considerably among the four global polypropylene vendors tested. Notably, the best-performing polypropylene tubes still introduced 847 labware-derived contaminant m/z values across three manufacturing batches, while less suitable polypropylene microcentrifuge tubes contributed thousands of contaminants per extraction.[1] These polypropylene-derived contaminants caused hundreds of instances of ion suppression of co-eluting endogenous lipids.[2] Even when such contaminants do not directly induce matrix effects, they may be misidentified as endogenous lipids. In total, 396 plastic-derived contaminant m/z values were found

within 10 ppm of COMP_DB lipid entries. Of particular concern, 21 primary amide and fatty acid surfactants—indistinguishable from endogenous biological lipids—severely hindered the accurate identification and quantification of their corresponding human serum lipid equivalents.[2]

We have developed the first lipidomics-tailored contamination library, systematically cataloguing contaminants originating from glassware, plasticware, extraction and analysis solvents. The database was constructed using a dedicated computational pipeline integrating peak alignment, peak extraction, and spectrum clustering, and its versatility was validated across multiple MS platforms and vendors. Delivered through an open-access, user-friendly web application, this resource enables rapid contaminant identification from m/z values and MS/MS spectra. Our goal is to empower researchers to reliably distinguish contaminants from true lipids, thereby improving the accuracy, reproducibility, and overall data quality of untargeted lipidomic workflows.

References

1 Canez, C. R.; Li, L. Anal Chem. 2024, 96 (8), 3544-3552.

2 Canez, C. R.; Li, L. Anal Chem. 2024, 96 (21), 8373-8380.

References

[1] Canez, C. R.; Li, L. Anal Chem. 2024, 96 (8), 3544-3552.
[2] Canez, C. R.; Li, L. Anal Chem. 2024, 96 (21), 8373-8380.

Development of a Serum-Based lipid-protein Diagnostic Assay for the Early Detection of Ovarian Cancer

Erin Han (1), Charles Nichols (2), Moises Zapata (1), Rachel Culp-Hill (1), Brendand Giles (1), Vuna Fa (2), Liang Zhao (3), Kim Ekroos (4), Abigail McElhinny (1)

(1) AOA, (2) AOA Dx, (3) Complete Omics, (4) Lipidomics Consulting Ltd

Type of abstract: ORAL

Introduction and Aims:

Ovarian cancer (OC) is lethal. It remains the fifth-leading cause of cancer-related deaths among women. Most (80%) patients are diagnosed at late-stage (III/IV) disease, with five-year survival rates <30%. This is largely due to a few key factors. First, most OC patients seek care for symptoms at the earliest stages (I/II), but the symptoms are non-specific and overlap with several potential benign gastrointestinal and other gynecological conditions. This causes a long patient journey with the OC diagnosis requiring ~9 months on average in the US. Second, current diagnostic tools lack sensitivity and specificity for OC, underscoring the critical unmet need for innovative approaches. We are utilizing multi-omics to develop a sensitive, non-invasive blood-based assay for detection of early-stage OC when women first present with symptoms. Our published work (1) identified a discovery-based lipidomic profile in serum of patients with ovarian cancer. We determined that combining a few significant putative lipid features with well-known protein biomarkers and machine-learning resulted in reproducible models for distinguishing OC in the symptomatic population. Here, we describe verification of the top-performing putative lipid features and their migration to a targeted routine triple-quadrupole (QqQ) mass spectrometry (MS) method to enable quantification and further identification of key biological drivers for OC-specific signatures.

Material and Methods:
Our discovery-based untargeted high-resolution MS (HRMS) workflow identified potential lipid features that could enhance performance of known OC-protein biomarkers. Filtering of the discovery features was performed with a total of ~1000 features comprising the final feature space for ML-based modeling. The top-performing lipid features were identified for migration to QqQ-MS and further verification. Data dependent MS/MS methods were employed to improve the quality and number of feature identifications, with a resulting ~100 features obtained after down-selection.

Results:
QqQ MS method development was executed on a TQ-Absolute (Waters Corporation) using previous chromatographic conditions (1). Since the discovery workflow provided tentative and putative lipid identifications, the identification of the top-performing lipid features had first to be verified. Analyses such as HRMS and MS/MS in both polarity modes, retention time (RT) and lipid standard matching, and manual spectra evaluations were performed. For example, accurate mass, product ions of the head group and acyl anions, and expected RTs confirmed targets as phosphatidylcholines (PC). Utilizing this approach corrected wrongly identified features by the Compound Discover software used in the discovery workflow.
Identification of the correct precursors and product ions of each top-performing lipid feature permitted successful transfer of the discovery biomarker panel to the multiple reaction monitoring (MRM) QqQ MS platform. MRM conditions were optimized for each lipid biomarker to maximize signal response and achieve highest selectivity and sensitivity. Lipid class specific standards were included to similar levels as the endogenous lipid biomarkers, paired with relevant species, to permit quantification. High analytical performance of the developed targeted MRM method was achieved, with variations below 15% for most lipid biomarkers recorded.

Conclusions:
Our multi-omic approach determined that differences in lipid and protein abundance accurately distinguish ovarian cancer in non-cancerous controls representing the symptomatic population. Here, we thoroughly translated the putative discovery-based lipid features to verified targeted lipid biomarkers. We utilized mass accuracy, characteristic lipid fragmentation, and lipid standards, leaving minimal room for ambiguity for some putative identifications. Our approach permitted successful establishment of a robust targeted quantitative MRM method. Work now is in process to verify the method using a third independent clinical cohort and further refine our clinical diagnostic assay.

References

[1] Giles, B., Culp-Hill, R., et al. Cancer Research Communications (2025) 5 (9): 1516-1529. https://doi.org/10.1158/2767-9764.CRC-25-0140

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

Reusch Sabrina (1), Ludwig Anna (1), Ekroos Kim (2), Ejsing Christer S. (3), Huff Seth (4), Burkhardt Ralph (1), Gerhard Liebisch (1), Hoering Marcus (1)

(1) University Hospital Regensburg, (2) Lipidomics Consulting Ltd, (3) University of Southern Denmark, (4) Larodan AB

Type of abstract: ORAL

Integrative Lipidomics and Transcriptomics Identify Distinct Tumor Cell Subtypes in Human Colon Cancer

Ramón María Rodríguez (1), Albert Maimó-Barceló (2), Karim Pérez-Romero (3), Cristina Huergo (4), Teresa Ximelis (2), José Andrés Fernández (5), Gwendolyn Barceló-Coblijn (6)

(1) Health Reserch Institute of the Balearic Islands, Palma de Mallorca,, (2) 1Health Reserch Institute of the Balearic Islands, Palma de Mallorca, (3) Health Reserch Institute of the Balearic Islands, Palma de Mallorca, (4) 2University of the Basque Country, Leioa, (5) University of the Basque Country, Leioa, (6) Health Research Institute of the Baleric Islands (IdISBa)

Type of abstract: ORAL

Introduction

Colorectal cancer (CC) is one of the most preventable malignancies, yet it remains among the deadliest. Despite initial treatment success, up to 50% of patients experience relapse, often in the form of metastasis. The biological mechanisms driving recurrence are still poorly understood, underscoring the need for improved tumor classification. Spatially Resolved Omics technologies offer near single-cell resolution of molecular profiles, enabling the identification of cell-specific alterations. Within this framework, lipid species are emerging as promising biomarkers of malignancy.

Methods

CC patients undergoing surgery at Son Espases Hospital were recruited under approved ethics protocol (IB4568/21PI). Tissue sections (12 µm) were cryosectioned using a Leica CM3050S and mounted on slides. Spatial lipidomics (n=7) was performed using MALDI-MSI on ITO-coated slides with 1,5-diaminonaphthalene matrix sublimation (Ace Glass 8023). Scanning was conducted in negative-ion mode at 10 µm pixel resolution using the TIMSTOF-Flex system (Bruker), and data were analyzed with SCILS Lab (Bruker). Spatial transcriptomics (n=7) was carried out using the Visium platform (10× Genomics), following manufacturer instructions. Data were processed using Space Ranger and Loupe Browser software, with further analysis performed using Orange data mining software.

Results

Building on our previous work using MALDI-MSI to map lipid distribution in healthy colon tissue, adenomatous polyps, and carcinomatous sections, we confirmed that lipid profiles are highly specific to cell type and differentiation state, reinforcing their potential as biomarkers for pathophysiological conditions.

In this study, we focused on the tumor epithelium to identify lipidomic signatures associated with invasive potential. Using a spatially resolved multi-omic approach, we integrated spatial transcriptomics and lipidomics to correlate gene expression with lipid profiles in consecutive sections of advanced colon cancer tissue. This integrative analysis revealed dynamic interactions between gene expression and lipid metabolism, influenced by both tissue type and disease progression.

We identified distinct tumor epithelial regions based on transcriptomic signatures, which showed high spatial co-registration with region-specific lipidomic profiles. Further analysis revealed that these regions are dependent on enterocyte lineage and maturation state, as supported by gene set enrichment analysis and our previously established lipid species distribution linked to enterocyte maturation. We defined at least four subpopulations within the tumor epithelium: Stem, TA-enterocyte, TA-secretory, and Secretory. Each subpopulation exhibited a unique relationship between degree of phospholipid unsaturation, differential expression of genes involved in lipid metabolism and tumor malignancy.

Conclusion

Our spatially resolved multi-omic analysis provides new insights into the molecular heterogeneity of colon cancer, emphasizing the interplay between lipid metabolism and gene expression across distinct tumor epithelial regions. The detailed characterization of epithelial subpopulations highlights the potential of integrated lipidomic-transcriptomic profiling to uncover biomarkers and mechanisms linked to tumor progression and invasiveness. These findings lay the groundwork for

future studies into the functional roles of specific lipid species in cancer biology and may inform the development of targeted therapies based on tumor metabolic phenotypes.

Lipidomic Analysis of Plasma and Testicular Tissue for the Discovery of Novel Testicular Cancer Biomarkers

Inês Nobre (1), Helena Beatriz Ferreira, Inês M. S. Guerra, Marisa Pinho, Tânia Melo, Pedro Domingues, Artur Paiva, Pedro F. Oliveira, M. Rosário Domingues

(1) University of Aveiro

Type of abstract: POSTER

Introduction and Aims: The diagnosis and prognosis of testicular cancer remain hindered by the absence of robust and specific biomarkers. Although alterations in lipid metabolism have been extensively documented in various cancer types through lipidomic analyses, their application to testicular cancer is still limited.

Material and Methods: In this study, an untargeted lipidomic approach based on reverse-phase liquid chromatography coupled to mass spectrometry (C18 RP-LC-MS) was employed to unravel the variation of the lipid profile in cancer testicular tissue. We have evaluated the alterations in the lipidomic profile of testicular tissue (tumour and non tumoural tissue of the same patient, n=10) as well as in plasma from patients diagnosed with testicular cancer in comparison to healthy controls (n=10).

Results: Principal Component Analysis (PCA) demonstrated a pronounced separation between the sample groups. In malignant testicular tissue, there was a marked upregulation of some phosphatidylcholine (PC) and lysophosphatidylcholine (LPC) lipid species containing saturated (SFA) and monounsaturated fatty acyl chains (MUFA). In contrast, significant downregulation was observed in phosphatidylinositol (PI), phosphatidylserine (PS), and phosphatidylglycerol (PG) lipid species, most of which were esterified with polyunsaturated fatty acids (PUFA). Plasma lipid profiling revealed an increased levels of PC and LPC species in patients with testicular cancer, mirroring the tissue findings. Additionally, certain sphingomyelin (SM) species were upregulated, whereas various triacylglycerol (TG) and diacylglycerol (DG) species were significantly reduced. Five lipid species, TG 52:6, TG 56:7, PE P-40:3/PE O-40:4, PC 42:3 and PC P-37:2, showed the same variations in both matrices, suggesting their potential as biomarkers.

Conclusions: Overall, this work highlights distinct lipidomic signatures in testicular cancer and supports the use of comprehensive lipid profiling in the discovery of novel biomarkers that could aid in the clinical management of this malignancy.

References

[1] (1) Das S, Dey MK, Devireddy R, Gartia MR. Biomarkers in Cancer Detection, Diagnosis, and Prognosis. Sensors. 2023; 24(1):37. doi: 10.3390/s24010037. PMID: 38202898.

Analysis of changes in lipid content in aronia berry in different harvest years

Takuya Yamane, Ayaka Yamazaki, Momoko Imai, Masatomo Takahashi, Takeshi Bamba, Susumu Uchiyama

Type of abstract: POSTER

Direct lipid analysis of salivary exosomes and microvesicles by flow field-flow fractionation coupled with electrospray ionization tandem mass spectrometry

Dong Gyun Kim (1), Myeong Hee Moon (1)

(1) Yonsei University

Type of abstract: POSTER

This study aims to directly analyze lipids from saliva, a non-invasive and easily collectible biological fluid that imposes minimal stress on the patient, by utilizing flow field-flow fractionation on-line coupled with elecromspray ionization tandem mass spectrometry (FlFFF-ESI-MS/MS). Exosomes and microvesicles are lipid bilayer-enclosed vesicles present in various body fluids such as blood, urine, and saliva. They are composed of lipids, membrane proteins, and RNA. These vesicles play critical roles in intercellular communication and immune regulation, making them promising candidates for disease diagnosis. In particular, since cancer profoundly affects cellular metabolism, lipidomic profiling of these vesicles may provide insights into cancer-associated alterations. In this study, we isolated salivary exosomes and microvesicles from gastric cancer patients and analyzed their lipid profiles by (FlFFF-ESI-MS/MS without a separate lipid extraction followed by typical LC-ESI-MS/MS measurements. FlFFF is a size-based separation technique that operates without a stationary phase, using an open, unpacked channel and aqueous carrier liquid–making it highly suitable for analyzing sensitive biological particles including extracellular vesicles (EVs) by sizes without denaturation. Since the high outflow rate of the conventional FlFFF can lower the ionization efficiency, in this study, the analytical method was optimized by miniaturizing the channel and implementing a thickness-tapered FlFFF, which improved the early sample elution and recovery rate as well as the resolution. We successfully analyzed the lipid profiles of exosomes and microvesicles in saliva samples from patients with gastric cancer and healthy controls by FlFFF-ESI-MS/MS. This analysis provided a basis for the use of salivary exosome and microvesicle lipidomics in the discovery of gastric cancer biomarkers.

Lipid analysis of human skin with melanoma and dysplastic nevus by nanoflow UHPLC-ESI-MS/MS

Junha Choi (1), Soomin Kang (1), Ayse Caner (2), Myeong Hee Moon (1)

(1) Yonsei University, (2) Ege University

Type of abstract: POSTER

Melanoma is a highly malignant skin cancer with an increasing incidence. Its prognosis and survival rate are closely associated with the disease stage, emphasizing the need for early diagnosis and effective biomarkers. Dysplastic nevi (DN) resemble melanoma and may act as potential precursors, increasing the risk of disease progression. Lipids are essential biomolecules involved in cellular structure, energy storage, and signal transduction. Metabolic alterations can lead to changes in lipid profiles, making lipidomics a valuable tool for disease diagnosis and pathogenesis research. This study aimed to identify potential lipid biomarkers for the diagnosis of melanoma and its differentiation from DN by comparing their lipid profiles. Lipidomic analysis was performed on skin samples collected from patients with melanoma, DN, and benign nevi. Skin samples were obtained using a non-invasive tape-stripping method, and lipids were extracted using an optimized extraction protocol. Extracted lipids were analyzed using nanoflow ultrahigh-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry (nUHPLC-ESI-MS/MS). Comparative analysis of lipid profiles among groups revealed lipid species specifically altered in melanoma and DN, suggesting their potential as biomarkers for improved diagnostic accuracy.

Untargeted Lipidomics and Oxylipin Profiling Reveal Metabolic Alterations Induced by CPT1A Overexpression in a CCl_4 Mouse Model of Liver Fibrosis

Beatriz Linillos-Pradillo (1), Paula Cuevas-Delgado (1), Belén Sirera (2), Santiago Lamas (2), Coral Barbas (1)

(1) Centre for Metabolomics and Bioanalysis (CEMBIO), (2) Centro de Biología Molecular "Severo Ochoa" (CBMSO) (CSIC-UAM)

Type of abstract: POSTER

Introduction and Aims:

Non-alcoholic fatty liver disease (NAFLD) can progress to liver fibrosis, a complex process driven by oxidative stress, inflammation, and alterations in lipid metabolism. The enzyme carnitine palmitoyltransferase 1A (CPT1A), a key regulator of mitochondrial β-oxidation of fatty acids, may play a significant role in this pathological transition. However, the specific role of CPT1A in this process remains unclear, highlighting the need to thoroughly investigate changes in the lipid and oxylipin profile, key mediators of inflammation and fibrosis progression.

Material and Methods:

A murine model with CPT1A overexpression (Lap-CPT1A + DOXI) and liver injury induced by carbon tetrachloride (CCl_4) was used to assess lipid classes and oxidized lipids in both liver tissue and plasma, aiming to investigate local and systemic effects. An untargeted lipidomic analysis was performed using Liquid Chromatography coupled to Quadrupole Time-of-Flight Mass Spectrometry (LC-QTOF-MS) to obtain broad lipid coverage (1). Targeted oxylipins were quantified using Reverse-Phase Ultra-Performance Liquid Chromatography coupled to negative Electrospray Ionization and Triple Quadrupole Tandem Mass Spectrometry (RP-UPLC-ESI(−)-QqQ-MS/MS) (2). Univariate and multivariate statistics were performed along with the accurate annotation of lipids with statistically significant changes using software and after manual verification.

Results:

A reduction in nearly all lipid classes was observed in the liver of WT mice exposed to CCl_4, while plasma exhibited systemic hyperlipidemia, suggesting lipid release from the damaged liver into circulation. Under basal conditions, CPT1A

overexpression increased carnitine-related species (CAR) and decreased glycerophospholipids (GP) in plasma, while in the liver, most lipids increased, with specific signalling responses mediated by phosphatidylserine (PS) and phosphatidylcholine (PC). After CCl_4-induced injury, CPT1A overexpression led to a decrease in fatty acids (FA), PS, ceramides (Cer), and sphingomyelins (SM), and an increase in regenerative lipid species such as phosphatidylglycerol (PG) and phosphatidylinositol (PI). In plasma, CPT1A induced a specific signalling response and modulation of the lipid profile under CCl_4 challenge. Regarding oxylipins, no statistically significant changes were observed after CPT1A overexpression and CCl_4-induced damage.

Conclusions:

CPT1A may differentially modulate systemic and hepatic lipid metabolism by promoting β-oxidation, preserving mitochondrial integrity, suppressing inflammation, and potentially reversing systemic effects of CCl_4-induced liver injury. However, further studies are needed to clarify the precise role of CPT1A in hepatic fibrogenesis.

References

[1] (1) Gonzalez-Riano, C. et al. (2021). Journal of Chromatography Open, 1: 100018.
[2] (2) Camunas-Alberca, S. M. et al. (2025). Prog Neuropsychopharmacol Biol Psychiatry, 139: 111387. PMID: 40306479.

Unveiling cellular changes in leukaemia cell lines after cannabidiol treatment through lipidomics

David Chamoso-Sanchez (1), Martina Panini (2), Clarissa Caroli (3), Matilde Marani (2), Lorenzo Corsi (2), Francisco J Rupérez (4), Federica Pellati (5), Antonia Garcia (4)

(1) Centre for Metabolomics and Bioanalysis (CEMBIO), (2) Department of Life Sciences, University of Modena and Reggio Emilia, Via Giuseppe Campi 103-287, 41125, Modena, Italy., (3) Department of Life Sciences, University of Modena and Reggio Emilia, Via Giuseppe Campi 103-287, 41125, Modena, Italy. & Clinical and Experimental Medicine PhD Program, University of Modena and Reggio Emilia, Via Giuseppe Campi 287, 41125, Modena, Italy, (4) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28660, Boadilla del Monte, Madrid, Spain., (5) Department of Life Sciences, University of Modena and Reggio Emilia, Via Giuseppe Campi 103-287, 41125, Modena, Italy

Type of abstract: POSTER

Adaptive lipidomic responses of the marine fungus Zalerion maritima to environmental stressors: a promising source of bioactive lipids

Felisa Rey (1), Joana Costa, Marisa Pinho, Inês M. S. Guerra, Maria A. Salvador, Tânia Melo, Stefano Bonciarelli, Laura Goracci, Rosário M. Domingues, Artur Alves

(1) Univeristy of Aveiro

Type of abstract: POSTER

Marine fungi have gained attention as promising producers of bioactive compounds [1] due to their adaptability to diverse and extreme environments, which shape their distinctive biochemical profiles. The marine fungus Zalerion maritima was cultivated at different temperatures (15, 20, and 30 °C) and salinities (0, 2.5, and 5% sea salt) to investigate how environmental conditions influence its lipidome and bioactive potential. Lipidomic profiling using C18-LC-HR-MS/MS identified 263 lipid species spanning 16 classes, including phospholipids, sphingolipids, neutral glycerolipids, and betaine lipids. Elevated temperature and salinity led to a notable reduction in lipid species containing the polyunsaturated fatty acid (PUFA) 18:3 n-3, suggesting a structural adaptation to maintain membrane stability. Fatty acid analysis by GC-MS revealed a high PUFA content, especially 18:2 n-6 and 18:3 n-3. Cultivation at 30 °C significantly decreased 18:3 n-3 levels while increasing 18:1 n-9, demonstrating lipid remodelling capacity under thermal stress. Suboptimal growth conditions enhanced the production of lipids with anti-inflammatory activity. Bioassays revealed the highest COX-2 inhibition at extreme salinities (0% and 5% sea salt), with inhibition levels of 74.7 ± 12.3% and 76.8 ± 12.2%, respectively. These results underscore the lipidome plasticity of Z. maritima and its potential as a sustainable source of high-value lipids for biotechnological applications.

References

[1] [1] Abraul, et al. (2023). Evaluation of lipid extracts from the marine fungi Emericellopsis cladophorae and Zalerion maritima as a source of anti-inflammatory, antioxidant and antibacterial compounds. Marine Drugs, 21, 199. https://doi.org/ 10.3390/md21040199

Exploring lipidome in Fragile X Syndrome

Laroui Asma (1), Robillard Isabelle (2), Ruiz Matthieu (2), Çaku Artuela (3)

(1) University of Sherbrooke, (2) Metabolomics Platform of the Montreal Heart Institute, (3) University of Sherbrooke.

Type of abstract: POSTER

Lipid biomarker discovery in plasma of patients with heart failure and type 2 diabetes undergoing metabolic correction with tirzepatide

NIKOLAY V. BEREZHNOY (1)

(1) Nazarbayev University, National Laboratory of Astana

Type of abstract: POSTER

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

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

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

Lipid Profile of Metabolic Dysfunction-Associated Steatotic liver disease (MASLD) Among People Living with HIV after HCV Eradication

Belén Paula Fernández Requena (1), Ana Virseda-Berdices (2), Juan Berenguer (3), Juan González-García (4), Carolina Gonzalez-Riano (5), Cristina Díez (6), Víctor Hontañón (7), Paula Muñoz-García (8), Amanda Fernández-Rodríguez (3), Coral Barbas (5), Salvador Resino (9), Rubén Martín-Escolano (3), María Ángeles Jiménez-Sousa (2)

(1) San Pablo CEU University, (2) Unidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología (CNM), Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain., (3) Centro de Investigación Biomédica en Red en Enfermedades Infecciosas (CIBERINFEC), Instituto de Salud Carlos III (ISCIII), Madrid, Spain., (4) ervicio de Medicina Interna-Unidad de VIH. Hospital Universitario La Paz. Madrid, Spain, (5) CEMBIO, Facultad de Farmacia, Universidad San Pablo CEU, Boadilla del Monte, Madrid, Spain., (6) Unidad de Enfermedades Infecciosas/VIH; Hospital General Universitario "Gregorio Marañón", Madrid, Spain., (7) Instituto de Investigación Sanitaria La Paz (IdiPAZ). Madrid, Spain., (8) Instituto de Investigación Sanitaria Gregorio Marañón (IiSGM), Madrid, Spain., (9) nidad de Infección Viral e Inmunidad, Centro Nacional de Microbiología (CNM), Instituto de Salud Carlos III (ISCIII), Majadahonda, Madrid, Spain

Type of abstract: POSTER

Introduction and Aims:
While hepatitis C virus (HCV) eradication typically halts liver damage, some individuals continue to experience liver disease progression. Metabolic dysfunction-associated steatotic liver disease (MASLD) has emerged as a potential driver of this ongoing injury [1]. This study investigates the plasma lipidomic signatures linked to MASLD in patients with advanced liver fibrosis or cirrhosis, coinfected with human immunodeficiency virus (HIV) and HCV, after achieving sustained virologic response (SVR).

Material and Methods:
A cross-sectional analysis was conducted on 52 HIV/HCV-coinfected patients. Plasma samples collected at one- and six-years post-SVR were analyzed using untargeted lipidomics via liquid chromatography-mass spectrometry (LC-MS). A lipid annotation workflow developed by our lab was used to perform the annotation of the lipid species present in the plasma matrix [2]. MASLD served as the primary outcome. Multivariate analysis using orthogonal partial least squares discriminant analysis (OPLS-DA) and generalized linear models (GLMs), with correction for multiple testing, was employed to identify lipid alterations associated with MASLD.

Results:
MASLD prevalence increased from 28.9% at one-year post-SVR to 44.8% at six years. OPLS-DA revealed 225 lipid species at year one and 167 at year six (VIP ≥ 1) that differentiated individuals by MASLD status. After adjusting for confounders, GLMs confirmed significant associations with 116 lipids at the first time-point and 49 at the second. Early lipid changes included elevated phosphatidylcholines (PC) and phosphatidylethanolamines (PE), alongside reductions in lysophosphatidylcholines (LPC) and lysophosphatidylethanolamines (LPE). At six years, LPCs remained notably altered, and triglyceride levels were significantly higher in individuals with MASLD.

Conclusions:
The persistence and progression of MASLD following HCV clearance suggests ongoing metabolic dysregulation. Shifts in lipidomic profiles over time support the need for continued metabolic and hepatic monitoring in HIV/HCV-coinfected patients, even after achieving SVR.

References

[1] (1) Berenguer J, et al. Hepatology. 2017;66(2):344-56.
[2] (2) Fernández Requena B, et al. Communications Biology 2024;7:45.

Enhancing Confidence Annotation in Lipidomics by implementing Rule-Based Annotation and Retention Time Modelling in LipidMS

Maria Isabel Alcoriza Balaguer (1), Celia Regal (1), Juan Carlos Garcia (1), Marta Roca (1), Agustin Lahoz (1)

(1) IIS La Fe

Type of abstract: POSTER

Introduction and Aims:

The increasing interest in understanding the role of lipids in cellular function and disease has driven a significant growth in the field of lipidomics. However, despite technological advances in mass spectrometry and data acquisition strategies, lipid identification remains as the most critical bottleneck in the lipidomics workflow. This challenge arises primarily from the structural complexity of lipids and the high number of isomers within and across lipid classes. As a result, the number of false annotations remains too high for most current lipid annotation tools, most likely due to inappropriate annotation of adducts or identification of in source fragments as intact species, among other reasons. Therefore, there is a need for improving data analysis tools to provide high-confidence annotations while maximizing coverage and structural resolution.

Material and Methods:

LipidMS is an open-source R-based package designed to overcome the challenges of lipid identification by implementing a rule-based annotation strategy that leverages known fragmentation patterns of lipid classes acquired by untargeted liquid chromatography-tandem mass spectrometry (LC-MS/MS) [1,2]. This approach is designed to prioritize data quality and annotation confidence over the total number of lipid identities proposed. LipidMS supports the simultaneous processing of full scan, data-dependent acquisition (DDA), and data-independent acquisition (DIA) modes. The annotation relies on fragmentation rules that have been manually curated and experimentally validated, reducing the incidence of false-positive identifications. In its upcoming release (v3.1) [1,2], LipidMS will incorporate a significant new feature: the integration of retention time (RT) rules and RT-based modeling into the annotation pipeline. This addition enables the systematic filtering of ambiguous or low-confidence annotations based on chromatographic behavior and allows the propagation of high-confidence annotations to structurally related lipid species. RT models are trained on confidently annotated lipids and then used to predict the expected RT of analogous species.

Results:

The application of rule-based annotation in LipidMS has demonstrated a notable reduction in false positives compared to tools that rely solely on spectral matching. Although this strategy results in a lower overall number of annotations, the proportion of correctly assigned lipid identities is significantly higher, leading to more reliable datasets for downstream biological interpretation. Furthermore, the integration of RT rules and modeling in the upcoming release is expected to increase lipidome coverage without compromising identification accuracy. The use of RT-trained models enables the propagation of confident annotations to related lipids that might otherwise be missed, thereby enhancing both the depth and reliability of lipid profiling results.

Conclusion:

By combining rule-based MS/MS annotation with RT modeling, LipidMS presents a hybrid approach that not only increases the confidence of lipid annotations but also enhances annotation coverage, particularly for lipids that fall outside existing spectral libraries, which provides a more robust and biologically meaningful interpretation of lipidomics datasets.

Keywords: Lipidomics; LC-MS; bioinformatics

References:

(1) Alcoriza-Balaguer MI, et al. (2019) Anal Chem, 91(1):836-845.PMID: 30500173

(2) Alcoriza-Balaguer MI, et al. (2022) Bioinformatics, 38(20):4826-4828.PMID: 36005855.

References

[1] Alcoriza-Balaguer MI, et al. (2019) Anal Chem, 91(1):836-845.PMID: 30500173
[2] Alcoriza-Balaguer MI, et al. (2022) Bioinformatics, 38(20):4826-4828.PMID: 36005855.

LipidMS Shiny app: Improving Lipid Annotations through Retention Time Modeling in Lipidomics

Celia Regal Marqués (1), María Isabel Alcoriza Balaguer (1), Marta Isabel Roca Marugan (1), Juan Carlos García Cañaveras (1), Agustín Lahoz Rodríguez (1)

(1) IIS La Fe

Type of abstract: POSTER

Introduction and Aims:

Liquid chromatography (LC) coupled with high-resolution mass spectrometry (HRMS) is the most prominent analytical technique used for untargeted lipidomics. Lipid annotation relies on the acquisition of MS/MS information coupled to spectral matching or the use of fragmentation rules. However, the number of false annotations remains too high for most current lipid annotation tools, most likely due to inappropriate annotation of adducts or identification of in source fragments as intact species, among other reasons. We hypothesize, that the use of the retention time (RT) variable during the annotation process might contribute to improve lipid annotation, particularly for those species where the MS/MS information does not provide sufficient level of evidence to assign an identification.

Material and Methods:

New functions were designed and integrated into the R package LipidMS (1, 2) with the aim of improving the quality of lipidomic analysis by incorporating RT as an additional variable in the annotation process. These functions operate in two complementary phases. First, an automated filtering step is applied to remove unreliable annotations generated by LipidMS. To achieve this, lipid classes potentially involved in in-source fragmentation are analyzed using clustering based on RT, carbon number (CN), and double bonds (DB), identifying RT overlaps between classes (e.g., between lysophospholipids and phospholipids) and retaining only non-overlapping groups. Then, within each lipid class, species that deviate from the expected RT trend based on their structural characteristics (CN, DB) are removed, resulting in a refined set of high-confidence annotations. In the second phase, RT is modeled for each lipid class as a function of CN and DB, allowing the prediction of RT values for new lipid candidates generated from their m/z values. This enables the proposal of additional annotations for previously unassigned features, provided there is concordance between the measured m/z and the predicted RT. In parallel, a Shiny application was developed to enable interactive visualization and inspection of the results, allowing users to manually curate annotations by removing false positives not detected automatically or restoring discarded annotations that are deemed valid upon visual inspection, thereby offering greater flexibility and control over the final quality of the analysis.

Results:

The implementation of the new functionalities in LipidMS was evaluated on multiple real datasets, demonstrating an average 20 % removal of inconsistent annotations through automatic filtering based on RT, CN and DB. At the same time, RT prediction for lipid candidates yielded an additional 15 % of identification proposals whose m/z and predicted RT closely matched the expected values for their respective classes. Thus, the total number of annotations remained similar to the original, but with a substantial improvement in overall reliability. The accompanying Shiny app provides a straightforward interface for reviewing and manually adjusting annotations, enabling users to discard false positives or restore valid identities with just a few clicks. This expert validation layer proves critical for noisy samples or those with limited reliable references, reinforcing the quality and confidence of the final results. These findings underscore the potential of this strategy to enhance both the reliability and depth of lipid annotation, laying a solid foundation for future extensions aimed at exploring more complex metabolic dynamics.

Conclusion:

Integrating RT modelling into the LipidMS workflow markedly reduces false annotations and enables the proposal of new lipid candidates corroborated by both m/z and RT information. Available now via the LipidMS Shiny app, this enhancement delivers a more reliable, visual and user-friendly annotation process, especially when MS/MS evidence alone is insufficient.

References

[1] (1) Alcoriza-Balaguer MI, et al. (2019) Anal Chem, 91(1):836-845.PMID: 30500173
[2] (2) Alcoriza-Balaguer MI, et al. (2022) Bioinformatics, 38(20):4826-4828.PMID: 36005855.

Lipid Signatures in Urinary Extracellular Vesicles as Non-Invasive Biomarkers in Alcohol Use Disorder

Blanca Martín Urdiales (1), Carla Perpiñá-Clérigues (2), Susana Mellado (3), María Pascual (3), Francisco García García (2)

(1) Centro de Investigación Príncipe Felipe (CIPF), (2) Príncipe Felipe Research Center, (3) University of Valencia

Type of abstract: POSTER

Lipids have proven to be valuable biomarkers in many diseases due to their critical involvement in physiological and pathological processes. In this context, urine-derived extracellular vesicles (EVs) have emerged as a promising, non-invasive source for lipid biomarker discovery, offering significant advantages for disease diagnosis and monitoring. Based on the hypothesis that the lipid profile of urine EVs can reveal specific biomarkers associated with chronic alcohol consumption and its systemic effects, this study aimed to identify differential lipid species in patients with alcohol use disorder (AUD). Urine and plasma samples were collected from male patients diagnosed with AUD. Urine EVs were isolated and subjected to a highly sensitive lipidomic analysis using mass spectrometry, followed by bioinformatic exploration of differential lipid abundance and enzymatic activity related to lipid metabolism. Our results identified fatty acid 22:0 (FA 22:0) as a potential biomarker, consistently detected in both urine and plasma of AUD patients. Additionally, we observed an increased saturation of acyl chains and elevated levels of long-chain fatty acids (22-24 carbons), lipid alterations that have been linked to inflammation, metabolic dysfunction, and cancer risk associated with AUD. In conclusion, this study highlights the potential of urine EV lipid profiling as a non-invasive strategy for biomarker discovery in AUD, providing new insights for disease assessment and future clinical applications.

Comprehensive Lipidomic Characterization of Livers in APP/PS1 Transgenic Mice: Comparison of Profiles Between Treated and Untreated Mice

Vladimír Vrkoslav (1), Štěpán Strnad (1), Andrea Pačesová (1), Anna Mengr (1), Veronika Strnadová (1), Blanka Železná (1), Jaroslav Kuneš (1), Lenka Maletínská (1)

(1) Institute of Organic Chemistry and Biochemistry of the Czech Academy of Sciences

Type of abstract: POSTER

Introduction and Aims:

Alzheimer's disease (AD) is a complex neurodegenerative disorder characterized not only by central nervous system pathologies such as β-amyloid (Aβ) plaques and Tau neurofibrillary tangles, but increasingly by systemic metabolic impairments. Metabolic dysfunction-associated steatotic liver disease (MASLD) has gained attention for its potential role in modulating the pathogenesis of AD through its contribution to systemic insulin resistance, lipid dysregulation, and peripheral inflammation. We investigated the role of MASLD in the context of AD-like pathology using the APP/PS1 transgenic mouse model of amyloidosis and wild-type (WT) controls, both fed a high-fat diet (HFD) to induce obesity, insulin resistance, and hepatic steatosis[1]. This study is focused on comparing liver lipidome profiles across six well-defined groups of HFD-fed mice differing by genotype and treatment - the lipidized prolactin-releasing peptide analog Palm^{11}-PrRP31, or the GLP-1 receptor agonist liraglutide.

Material and Methods:

Nine-month-old male APP/PS1 transgenic mice and age-matched wild-type (WT) control mice on a C57BL/6 background were used in this study. Each genotype was further divided into three pharmacological treatment groups. This generated six experimental cohorts: WT-HFD-saline, WT-HFD-Palm11-PrRP31, WT-HFD-liraglutide, APP/PS1-HFD-saline, APP/PS1-HFD-Palm^{11}-PrRP31, and APP/PS1-HFD-liraglutide, each group treated for 3 months subcutaneously once daily (excluding weekends) with mentioned peptides. Approximately 15 mg of wet liver tissue (n = 5 per group) was used for untargeted lipid extraction, employing the BUME (butanol-methanol) extraction protocol. Lipids were analyzed using an untargeted ultra-performance liquid chromatography coupled with electrospray ionization mass spectrometry (UPLC/ESI-MS) approach.

Results:

The data evaluation focused on comparing the lipidomes of APP/PS1 and WT mice under HFD conditions, with and without pharmacological treatment. Most annotated lipids belonged to the triacylglycerol (TG) and glycerophospholipid classes. Livers of HFD-fed mice showed elevated lipid levels, primarily TGs. The HFD affected APP/PS1 mouse livers more severely than those of WT mice. Treatment led to a reduction in TG and diacylglycerol (DG) species in liver tissue, with liraglutide being more effective at decreasing their concentrations compared to PrRP31 analog. Treatment led to an increase in certain phospholipid species - specifically some phosphatidylcholine (PC), phosphatidylethanolamine (PE), and phosphatidylserine (PS). Interestingly, some phosphatidylinositol (PI) and cardiolipin (CL) species were upregulated in APP/PS1 livers but downregulated in WT livers after liraglutide treatment.

Conclusions:

The concentration of TGs in the liver was significantly reduced in treated mice. The overall condition of liver tissue improved, indicating that both liraglutide and PrRP31 analog are active compounds with therapeutic potential against MASLD in this preclinical model.

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.

References

[1] Mengr A, et al. (2023) Nutrients, 3690, PMID: 37686722

Fattening Up the Facts: How Diet Can Improve Lamb Muscle Lipid Profiles

Beate Fuchs (1), Dannenberger Dirk (1), Lynda Perkins (2), Nigel Scollan (2)

(1) FBN Dummerstorf, (2) Institute for Global Food Security, Queen's University Belfast

Type of abstract: POSTER

Detection Limits of Oxidized Bulk Phospholipids in Biological Samples Using Conventional Matrices and 1-Pyrenebutyric Hydrazide in MALDI-TOF MS

Patricia Prabutzki, Jürgen Schiller, Johanna W. Schubarth, Kathrin M. Engel (1)

(1) Leipzig University

Type of abstract: POSTER

Oxidized lipids are involved in many severe diseases, associated with a dysregulated lipid metabolism and/or low-level chronic inflammation. An increase in reactive oxygen species, due to redox imbalance, leads to the generation of various lipid peroxidation products, including lysolipids and truncated carbonyl compounds, particularly carboxylic acids and aldehydes. The latter can readily react with other biomolecules, such as DNA or proteins and thereby impair their biological functions. Despite the growing interest in the role and function of oxidized lipids, their analysis remains challenging. This is due to several factors affecting their straightforward analysis, including their low abundance, their structural diversity and their transient nature as well as method-specific factors such as the impact of matrix-assisted laser desorption/ionization (MALDI) matrices on the detectability of such oxidized lipids.

Here, we evaluate the detectability of different oxidized phospholipids in rat liver extracts as the biological matrix, using different MALDI matrices including the well-known 2,5-dihydroxybenzoic acid and 9-aminoacridine as well as the newly introduced 4-(dimethylamino)cinnamic acid. We will also show that 1-pyrenebutyric hydrazide is a suitable matrix compound for the MALDI mass spectrometric (MS) analysis of native and oxidized lipids, as it can function both, as a derivatization agent for truncated oxidized lipids as well as a regular (UV)-MALDI matrix.

Comprehensive Lipidomic Analysis of Human Blood by Ultrahigh-Performance Supercritical Fluid Chromatography-Mass Spectrometry

Petra Peroutková (1), Robert Jirásko (1), Michal Holčapek (1), Ondřej Peterka (1)

(1) University of Pardubice

Type of abstract: POSTER

Introduction and Aims:

Lipidomics, the large-scale study of lipids and their biological roles, is crucial for understanding metabolic diseases and cellular function. Its application to human blood, particularly the analysis of both plasma and erythrocytes, is of growing importance in biomedical research. However, the distinct lipid compositions of these matrices, combined with the overall structural diversity and wide concentration range of lipids, present significant analytical challenges.

This study addresses these challenges by developing an optimised, robust, and high-throughput analytical workflow using ultrahigh-performance supercritical fluid chromatography coupled with mass spectrometry (UHPSFC/MS). The primary objective was to optimise and validate a sensitive and selective UHPSFC/MS method for comprehensive lipidomic analysis of human plasma and erythrocytes. Our work focused on method optimization to achieve superior separation efficiency and enhanced signal intensity, across multiple lipid classes, from non-polar to ionic lipid classes.

Material and Methods:

The modified MTBE extraction procedure was used for the sample preparation due to highest extraction recovery compared to other one- and two-phase extraction protocols. Ultrahigh-performance supercritical fluid chromatography was connected to high-resolution mass spectrometer and the bioinert Acquity Premier BEH HILIC (100 × 2.1 mm, 1.7 µm) column was employed for the separation of lipid (sub)classes. The gradient elution was used with a total run time of 7.5 min.

Results:

The new UHPSFC/MS method was developed based on the 80 standards from 45 lipid subclasses. The application of bioinert technology resulted in significantly improved peak shapes and increased sensitivity, especially for lipid classes containing phosphate group. Based on the comparison of common extraction procedures (BuMe, Folch, Bligh-Dyer, and MTBE) in lipidomic analysis, MTBE extraction provided the best results. Careful optimisation of the additive composition and pH of the aqueous phase achieved high extraction recovery for all investigated lipid classes. The optimised UHPSFC/MS method enabled the identification of hundreds of lipid species in human blood.

The method was rigorously validated following recommendation for bioanalytical methods, assessing calibration curves, detection and quantification limits, matrix effect, precision, accuracy, carry-over, and selectivity. Quantification was performed using internal standards for each lipid class and concentrations were calculated by laboratory-made LipidQuant 2.1 using both type I and type II isotopic corrections. Moreover, the method accuracy was confirmed using the NIST Standard Reference Material (SRM) 1950 Human Plasma, as the determined concentrations were in agreement with the consensus values from the ring trials.

Conclusions:

This UHPSFC/MS method represents a robust, reproducible, rapid, and sensitive approach for advanced lipidomic research. The results demonstrate the method's capability to resolve and quantify a significantly larger number of lipid species compared to the previous approach. Furthermore, we identified distinct lipidomic signatures between plasma, which primarily serves as a transport medium, and erythrocytes, which are a key component of blood cells with unique membrane compositions. Its successful application to complex biological samples such as human plasma and erythrocytes demonstrates its utility for future clinical research, such as investigating lipid-related biomarkers.

This work was supported by project no. CZ.02.01 .01/00/22_008/0004644 - Saving lives through research in early cancer detection and prevention: Molecular, genomic and societal factors (SALVAGE).

Multiplatform high-resolution metabolomics and lipidomics reveals etiology specific biomarkers in community acquired pneumonia

Henrique Santos-Oliveira (1), Miguel Fernández-García (2), Txema Marimón (3), Loreto Vidaur (3), Antonia García (2)

(1) Universidad San Pablo-CEU, CEU Universities, (2) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, 28660 Boadilla del Monte. España, (3) Hospital Universitario Donostia-Instituto de Investigación Sanitaria Biogipuzkoa, Paseo Dr. Begiristain, 20014 San Sebastián, Gipuzkoa. España

Type of abstract: POSTER

Introduction and Aims: Pneumonia is a prevalent acute respiratory infection involving the alveoli and distal airways, representing a significant global hearth burden and being associated with substantial morbidity as well as both short- and long- term morality across all age groups. It is mainly caused by viral, bacterial, or mixed infections, and leads to severe lung inflammation and damage. Accurate diagnosis guides targeted therapy, enhancing results and reducing side effects. Favorable patient outcomes rely on early and appropriate treatment, which depends on the timely distinction between bacterial and viral pneumonia.

Metabolomics has been employed as an approach for the identification of diagnostic biomarkers for infectious diseases.1 Infections caused by different pathogens trigger specific metabolic reactions, allowing metabolomics to identify unique biomarkers linked to disease etiology.2

Material and Methods:

This study is focused on identifying potential diagnostic biomarkers by characterizing the metabolite profiles of 23 bronchoalveolar lavage (BAL) samples collected from pneumonia patients in an intensive care unit. BAL samples were classified into four groups according to patient pneumonia etiology: bacterial (B, n = 8), viral (V, n = 4), coinfected (C, n = 6), and undetectable pathogen (S, n = 5). BAL sample extract analyses were performed applying a multiplatform workflow combining metabolomics and lipidomics. We applied reversed-phase liquid chromatography coupled to quadrupole-time-of-flight mass spectrometry (LC-QTOF/MS) for lipidomics and gas chromatography coupled to time-of-flight mass spectrometry (GC-TOF/MS) preceded by chloroformate derivatization for metabolomics, allowing the parallel measurement of lipids and volatile organic compounds, respectively. MS and MS/MS data were pretreated and reprocessed after analysis. One of the main difficulties of BAL samples is the need for normalization derived from the unknown amount of the collected biological samples. Lipid were annotated by preliminary software assisted annotation followed by manual evaluation of relevant pieces of information (m/z tolerance, MS/MS data, adduct profile, and retention time). Polar metabolome compounds were analyzed by GC-QTOF/MS, deconvoluted and searched against an in-house (accurate mass) and commercial libraries (NIST 2017) using spectral and retention index matching. Group comparison was performed by univariate and multivariate analysis.

Results: We annotated 404 different lipid molecular species by LC-QTOF/MS and polar 60 metabolites by GC-TOF/MS. Multivariate analysis showed that the metabolic profile of unaffiliated pneumonia patients was similar to that of viral pneumonia patients. We also observed that patients with bacterial-viral coinfection pneumonia tended to exhibit a metabolic profile more similar to that of bacterial pneumonia patients. This suggests bacterial infection to be the main driver of the metabolite alterations found during coinfection. ROC curve analyses reveal metabolite ratios with high discriminatory capacity (AUROC > 0.8) like glutaric acid/citric acid and citric acid/docosahexaenoic acid ratios (B vs V) and aconitic acid/citric acid (C vs V). This may be a result of the distinct metabolic responses that are induced by bacterial and viral pathogens during infection, which can be reflected in the levels of citric acid and other metabolites from fatty acid and TCA metabolic pathways.

Conclusions: This pilot study suggests that bacterial pneumonia causes metabolite alterations that can be used to distinguish between the etiology of V and C patients, pointing to potential biomarkers for classification of pneumonia according to etiology. These findings warrant further investigation with independent larger cohorts.

References

[1] (1) Globisch, D., Moreno, A. Y., Hixon, M. S., Nunes, A. A. K., Denery, J. R., Specht, S., Hoerauf, A., & Janda, K. D. (2013). Onchocerca volvulus-neurotransmitter tyramine is a biomarker for river blindness. Proceedings of the National Academy of Sciences of the United States of America, 110(11), 4218-4223.
[2] (2) Mehta, R., Chekmeneva, E., Jackson, H., Sands, C., Mills, E., Arancon, D., Li, H. K., Arkell, P., Rawson, T. M., Hammond, R., et al. (2022). Antiviral metabolite 3′-deoxy-3′,4′-didehydro-cytidine is detectable in serum and identifies acute viral infections including COVID-19. Med, 3, 204-215.e6. https://doi.org/10.1016/j.medj.2022.04.004

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

Óscar Pastor (1), Bohdan Babiy (2), E. Esplugues (3), S. Murray (4), Álvaro Hidalgo (2), Yanara Ferrer (2), Silvia Sacristán (2), D. Sainz de Urturi (3), I. Ruz-Maldonado (3), P. Fernandez-Tussy (5), Y. Suarez (3), C. Fernandez-Hernando (3)

(1) Hospital Universitario Ramón y Cajal-IRYCIS. Universidad de Alcalá (UAH), (2) Hospital Universitario Ramón y Cajal-IRYCIS, (3) Yale University School of Medicine, (4) Ionis Pharmaceuticals, INC., (5) Hospital Universitario Ramón y Cajal

Type of abstract: POSTER

Transcriptomic and lipidomic insights into lipid metabolism dysregulation in ELOVL1-deficient fibroblasts

Oliwia Lange-Andrzejewska (1), Agata Zwara (1), Agnieszka Jakubiak (2), Aleksandra Hliwa (3), Andrzej Dziembowski (4), Rafał Płoski (5), Adriana Mika (6)

(1) Department of Environmental Analysis, Faculty of Chemistry, University of Gdansk, Gdansk, Poland, (2) Tri-City University Animal House – Research Service Centre, Medical University of Gdansk, Gdansk, Poland, (3) Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdansk, Gdansk, Poland, (4) Institute of Biochemistry and Biophysics of the Polish Academy of Sciences, Warsaw, Poland, (5) Department of Medical Genetics, Medical University of Warsaw, Warsaw, Poland, (6) Department of Environmental Analysis, Faculty of Chemistry, University of Gdansk, Gdansk, Poland; Department of Pharmaceutical Biochemistry, Faculty of Pharmacy, Medical University of Gdansk, Gdansk, Poland

Type of abstract: POSTER

Background: ELOVL1 is an essential elongase for the synthesis of very-long-chain fatty acids, which are crucial for lipid metabolism and membrane integrity. Mutations in ELOVL1 are associated with severe lipid dysregulation. Despite the increasing interest in the role of ELOVL1 in lipid metabolism, integrative transcriptomic and lipidomic analyses in cellular models remain limited. This study aimed to investigate the effects of ELOVL1 deficiency on lipid-related gene expression in fibroblasts and the lipid profile of mouse tail skin.

Methods: For the study, the tails of 6-month-old mice were examined, and fibroblasts were obtained from them. RNA-seq was performed on fibroblasts isolated from wild-type (WT) mice (n=3) and ELOVL1-deficient mice: Homozygotes (HM) (n=3) and Heterozygotes (HT) (n=3). Differential gene expression was analysed using DESeq2, followed by gene ontology enrichment. Lipidomic profiling of tail skin samples (WT, n=23; HT, n=28; HM, n=20) was performed by LC-MS on an Orbitrap system and included the main lipid groups. Transcriptomic and lipidomic data were integrated using principal component analysis (PCA).

Results: PCA showed clear clustering of WT, HT, and HM samples based on both transcriptomic and lipidomic profiles. ELOVL1 deficiency led to extensive remodelling of the transcriptome with 2027 differentially expressed genes. The pathway enrichment revealed significant alterations in lipid metabolic processes and the cellular response to lipids. Dysregulated lipid-related genes included LCLAT1, PIK3C2A, Cyp1b1, CAV2, CLOCK, SACM1L, PTGS2 (COX-2), and FAR1, indicating a profound disruption of lipid homeostasis. Lipidomic profiling revealed decreased levels of very long-chain lipids (e.g., SM(d17:1_24:1), Hex1Cer(d18:1_24:1), PC(18:3_24:0)) and increased levels of long-chain lipids (e.g., SM(d17:1_22:0), Hex1Cer(t18:0_18:1), PC(21:0_18:1)) in ELOVL1-deficient mice.

Conclusions: The combination of transcriptomics and lipidomics reveals a novel link between ELOVL1 and lipid homeostasis in fibroblasts, and provides insight into the molecular mechanisms underlying lipid dysregulation associated with ELOVL1 mutations. These results provide new insights into the molecular mechanisms of lipid dysregulation associated with impaired fatty acid elongation.

Exploring skin lipidomics and lipid delivery with DESI-XS

Aina Millan (1), Noèlia Loza (2), Gabriela Fernanda Castelo (1), Ángela Hernández (3), Olga López (1)

(1) Institute for Advanced Chemistry of Catalonia, (2) Bicosome, S.L, (3) Hospital Infantil Niño Jesús

Type of abstract: POSTER

Desorption electrospray ionization (DESI) mass spectrometry is a powerful surface ionization method that enables the direct analysis of metabolites and lipids from complex biological samples without prior extraction or labeling. In dermatological research, where lipid composition is central to skin barrier integrity, DESI offers an attractive opportunity to probe both physiological and pathological processes. Here, we explored the potential of DESI-XS for skin research through two proof-of-concept studies.

In the first experiment, Sebutape® samples were collected from the chest of patients with ichthyosis, a group of rare genetic disorders characterized by defective skin barrier function, and from matched healthy controls. Tapes were mounted on microscope slides with double-sided adhesive and analyzed by DESI-XS under a methanol\water\formic acid (98:2:0.1) solvent system, with a scanning speed of 1 µm/min. Initial spectra revealed distinct molecular profiles between patients and controls, indicating alterations in the skin surface lipidome. Although definitive lipid identification is pending, these results highlight the promise of DESI-XS as a rapid and minimally invasive strategy to profile disease-specific lipid signatures directly from the skin. Such an approach could ultimately facilitate biomarker discovery and treatment monitoring in small patient cohorts, although throughput currently limits its scalability for larger studies.

The second study examined the penetration of two nanostructured lipid delivery systems—bicelles and lamellar body mimetics (LBms)—each incorporating ceramide IIIB, a key epidermal lipid involved in barrier organization. Both systems were applied to ex vivo porcine skin for 24 hours, after which sequential tape strips were collected and analyzed by DESI-XS. The technique enabled detection of the nanostructure components DPPC, DHPC, and ceramide IIIB, across different depths of the stratum corneum. Bicelles displayed a gradual, layer-by-layer penetration pattern, while LBms showed a more heterogeneous distribution. Importantly, both formulations delivered ceramide IIIB into deeper epidermal layers, supporting their potential use in therapeutic strategies aimed at restoring barrier lipids in disorders such as ichthyosis.

Overall, these proof-of-concept studies demonstrate the utility of DESI-XS as a label-free, high-resolution platform for investigating both endogenous skin lipid composition and exogenous lipid delivery. Despite current limitations related to solvent consumption and sample throughput, the technique offers unique advantages in terms of minimal sample preparation and spatially resolved lipid analysis. Future applications may include the identification of lipid biomarkers for ichthyosis, evaluation of novel delivery systems, and monitoring of therapeutic interventions in dermatological research.

Potential role of very long chain fatty acids in the development of early vascular ageing

Adriana Mika, Aleksandra Hliwa-Ślazyk (1), Magda Narajczyk (2), Magdalena Dzitkowska-Zabielska (3), Marika Franczak (4), Tomasz Śledziński (1), Krzysztof Narkiewicz (3)

(1) Department of Pharmaceutical Biochemistry, Medical University of Gdansk, Gdansk, Poland, (2) Bioimaging Laboratory, Faculty of Biology, University of Gdansk, Gdansk, Poland, (3) Department of Hypertension and Diabetology, Medical University of Gdansk, Gdansk, Poland, (4) Department of Biochemistry, Medical University of Gdansk, Gdansk, Poland

Type of abstract: POSTER

Background: Early vascular aging (EVA) syndrome refers to the premature decline in vascular function and structure that occurs at a faster rate than typically expected with normal aging [1]. Several factors can contribute to the development of EVA, including hypertension, genetic predispositions, diabetes, obesity, smoking, diet, and a sedentary lifestyle [2]. Previous studies assessing the relationship between lipids and EVA focused on cholesterol and triglycerides [3-5], and overlooked the role of very long chain fatty acids (VLCFAs) in EVA, despite their potential impact on cardiovascular health [6]. VLCFAs are usually defined as FAs with a carbon chain length of 20 or more and can be both ingested with food and synthesised in vivo [7]. The endogenous elongation of FAs to VLCFAs occurs in the endoplasmic reticulum, where the condensation step is catalysed by one of the seven elongases (ELOVL1-7), which have different substrate preferences [7]. Their concentrations are disturbed in various pathological conditions, including cancer [7-9], Hashimoto's thyroiditis [10] or liver cirrhosis [11]. Therefore, this study aimed to evaluate the fatty acid (FA) profile in EVA patients and to investigate the effects of EVA-induced FA profile changes on human aortic smooth muscle cells.

Methods and results: The FA profile in the serum of EVA patients was analysed by gas chromatography-mass spectrometry (GC-MS). The uptake of a very long-chain FA (VLCFA), the most elevated FA in the serum of EVA patients, into cell membranes was investigated in vitro in human aortic cells after VLCFA supplementation by differential centrifugation and subsequent GC-MS analysis. The morphology of human aortic smooth muscle cells after VLCFA supplementation was analysed by electron microscopy. Mitochondrial respiration was assessed using the Seahorse instrument. VLCFAs, including 22:0, 24:0, 26:0, 28:0, 30:0, and 32:0, were significantly increased in the serum of EVA patients with dyslipidemia ($p<0.001$), whereas 26:0 and 28:0 were increased in both groups of EVA patients with and without dyslipidemia ($p<0.001$). The addition of 26:0 and 28:0 to in vitro cultures of human aortic smooth muscle cells increased the thickness of the cell membrane ($p<0.001$) and impaired mitochondrial function ($p<0.05$). In addition, the number of mitochondria significantly increased in VLCFA-treated cells compared to untreated cells ($p<0.001$).

Conclusions: The serum VLCFA levels were increased in EVA patients, even in the absence of dyslipidemia. VLCFAs are incorporated into both outer and mitochondrial membranes and increase their thickness. In addition, VLCFAs impair mitochondrial respiration.

Translational perspective. This study provides evidence for the important and previously unrecognised role of VLCFAs in the pathogenesis of EVA. The uptake of VLCFAs, particularly 26:0 (hexacosanoic acid) and 28:0 (montanic acid), can lead to increased membrane thickness and impaired mitochondrial function. These changes can impair vascular elasticity, leading to arterial stiffness and increased cardiovascular risk. The exclusion of VLCFA from the diet and/or inhibition of their synthesis may be a potential new therapeutic strategy in the treatment of EVA patients.

References

[1] Bruno RM, Nilsson PM, Engström G, Wadström BN, Empana JP, Boutouyrie P, Laurent S. Early and Supernormal Vascular Aging: Clinical Characteristics and Association With Incident Cardiovascular Events. Hypertension 2020;76:1616-1624.
[2] Nilsson PM. Early vascular ageing - A concept in development. Eur Endocrinol 2015;11:26-31.
[3] Kiliç A, Baydar O, Elçık D, Apaydin Z, Can MM. Role of dyslipidemia in early vascular aging syndrome. Turkish J Med Sci 2021;51:727-734.
[4] Baba M, Maris M, Jianu D, Luca CT, Stoian D, Mozos I. The Impact of the Blood Lipids Levels on Arterial Stiffness. J Cardiovasc Dev Dis 2023;10.
[5] Gomez-Sanchez M, Gomez-Sanchez L, Patino-Alonso MC, Cunha PG, Recio-Rodriguez JI, Alonso-Dominguez R, Sanchez-Aguadero N, Rodriguez-Sanchez E, Maderuelo-Fernandez JA, Garcia-Ortiz L, Gomez-Marcos MA, Recio-Rodriguez JI, Martinez-Salgado C, Hernandez-Rivas JM, Gonzalez-Sarmiento R, Perez MB, Gonzalez-Sanchez J, Alonso-Dominguez R, Rodriguez-Martin C, Gomez-Sanchez M, Cabo-Laso A de, Sanchez-Salgado B, Mora-Simon S, Tamayo-Morales O, Agudo-Conde C, Lugones-

Sanchez C, Gonzalez-Porras JR, Bastida-Bermejo JM, Fuentes-Calvo I. Vascular aging and its relationship with lifestyles and other risk factors in the general Spanish population: Early Vascular Ageing Study. J Hypertens 2020;38:1110–1122.
[6] Miyazaki T, Shimada K, Hiki M, Kume A, Kitamura Y. High hexacosanoic acid levels are associated with coronary artery disease. Atherosclerosis 2014;233:429–433.
[7] Pakiet A, Ciosek M, Lange O, Duzowska K, Janczy A, Kapusta M, Razghonova Y, Ekman M, Abacjew-Chmyłko A, Kabata P, Mika A. Very long-chain fatty acids accumulate in breast cancer tissue and serum. Cancer Cell Int. 2025 Aug 4;25(1):296.
[8] Mika A, Kobiela J, Czumaj A, Chmielewski M, Stepnowski P, Śledziński T. Hyper-Elongation in Colorectal Cancer Tissue - Cerotic Acid is a Potential Novel Serum Metabolic Marker of Colorectal Malignancies. Cell Physiol Biochem. 2017;41(2):722-730.
[9] Zwara A, Hellmann A, Czapiewska M, Korczynska J, Sztendel A, Mika A. The influence of cancer on the reprogramming of lipid metabolism in healthy thyroid tissues of patients with papillary thyroid carcinoma. Endocrine. 2025 Jan;87(1):273-280. doi: 10.1007/s12020-024-03993-z.
[10] Hellmann A, Zwara A, Weryszko O, Czapiewska M, Korczyńska J, Sztendel A, Śledziński T, Mika A. Evaluation of the effect of Hashimoto's Thyroiditis on fatty acids involved in inflammation in the thyroid tissue. Biomed Pharmacother. 2025 Mar;184:117894. doi: 10.1016/j.biopha.2025.117894. Epub 2025 Feb 5.
[11] Hliwa A, Lange-Andrzejewska O, Laski D, Sledzinski M, Remiszewski P, Drobińska A, Mika A, Śledziński T. Alterations of the Fatty Acid Profile and the Expression of Genes Related to FA Metabolism in Cirrhotic Liver Tissue. Int J Mol Sci. 2024 Jul 25;25(15):8115.

Simultaneous characterization of queen bee-head lipids and pheromones using 4D-Lipidomics

Edward Rudt (1), Beixi Wang (2), Armando Alcazar Magaña (3), Alison McAfee (4), Leonard J. Foster (3), Erica Forsberg (2), Matthew R. Lewis (5)

(1) Bruker Daltonics GmbH & Co KG, (2) Bruker Scientific LLC, San Jose, California, (3) Michael Smith Laboratories, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada & Life Sciences Institute, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada, (4) Michael Smith Laboratories, Department of Biochemistry and Molecular Biology, University of British Columbia, Vancouver, BC, Canada & Department of Applied Ecology, North Carolina State University, Raleigh, NC, USA, (5) Bruker UK Ltd, Coventry, United Kingdom

Type of abstract: POSTER

Introduction

Bees are fundamental for maintaining biodiversity and ecosystem health, with their hives operating as highly organized social systems. The comprehensive analysis of lipids and pheromones in queen bee heads is critical for advancing our understanding of the biochemical foundations of social communication in honeybees. Queen pheromones act as indicators of reproductive status, shaping worker behavior and colony organization. Previous research has demonstrated that worker acceptance is more closely associated with age-dependent physiological changes than with direct reproductive metrics, underscoring the biological significance of this analytical approach [1].

However, the simultaneous characterization of lipids and pheromones from queen bee heads presents substantial analytical challenges due to the complexity and diversity of the molecular species involved. Bruker's novel timsMetabo platform addresses these challenges by combining the selectivity of trapped ion mobility spectrometry (TIMS) with high analytical sensitivity and broad mass range coverage, enabling precise and selective profiling of biologically and chemically complex samples. Here, we present an LC-TIMS-MS/MS approach developed to differentiate the pheromone and lipid profile of virgin and mated queen bee-heads.

Methods

Queen bee-head samples (virgin and mated) were homogenized and extracted using MTBE into lipid and metabolite fractions. These extracts were subsequently analyzed on the novel timsMetabo platform. Lipid profiling was performed using a Waters Acquity CSH C18 column with a total runtime of 18 min. Data acquisition was carried out based on the mobility range-enhancement (MoRE) acquisition mode in both positive and negative ionization modes. The acquired data was processed and analyzed in Bruker MetaboScape 2025b.

Results

The advanced analytical capabilities of the timsMetabo platform enabled comprehensive, rule-based annotation of diverse lipid classes, including phospholipids, sphingolipids, and neutral lipids. Furthermore, the implementation of the novel MoRE acquisition mode facilitated the simultaneous detection of pheromones by optimizing the transfer of low-mass metabolites alongside higher-mass lipids. The TIMS-enabled 4D-Lipidomics approach allowed for a detailed biochemical characterization of the queen bee-head samples from virgin and mated populations, indicating that maturity-dependent physiological changes in queen bees are reflected in the lipidome.

References

[1] McAfee, A., Magaña, A. A., Foster, L. J., & Hoover, S. E. (2024). Differences in honeybee queen pheromones revealed by LC-MS/MS: Reassessing the honest signal hypothesis. Iscience, 27(10).

Untargeted Lipidomics Identifies Pleiotrophin as a Regulator of Hepatic Lipid Metabolism with Sex-Specific Effects in a Mouse Model of Diet-Induced Obesity

Constanza Fernández Hernández (1), Héctor Cañeque Rufo (2), Luis Vivas Barreiro (1), Agata Zuccaro (3), Gonzalo Herradón (4), Francisco J. Rupérez (1), María Pilar Ramos Álvarez (3), Antonia García Fernández (1)

(1) 1 Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Boadilla del Monte, Madrid, Spain, (2) 2 Department of Chemistry and Biochemistry, Faculty of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Madrid, Spain 3 Department of Health and Pharmaceutical Sciences, Faculty of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Madrid, Spain, (3) 2 Department of Chemistry and Biochemistry, Faculty of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Madrid, Spain, (4) 3 Department of Health and Pharmaceutical Sciences, Faculty of Pharmacy, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, Madrid, Spain

Type of abstract: POSTER

Introduction and Aims

Obesity is a worldwide health issue linked to insulin resistance and metabolic syndrome, characterized by cardiovascular and metabolic alterations including hypertension, dyslipidaemia, hyperglycaemia, and hyperinsulinaemia. The liver plays a crucial role in regulating glucose and lipid homeostasis, wherein dysregulation of hepatic lipid metabolism contributes to insulin resistance development. A serious obesity-related complication is metabolic dysfunction-associated steatotic liver disease (MASLD), which ranges from simple fat buildup to more severe stage called metabolic dysfunction-associated steatohepatitis (MASH).

Recent investigations have identified pleiotrophin (PTN) as a heparin-binding cytokine involved in tissue repair, inflammation, signaling, and metabolic regulation. Building upon these findings, this study employed untargeted ultra-high-performance liquid chromatography-mass spectrometry with quadrupole time-of-flight (UHPLC-MS-QTOF) lipidomics approaches to comprehensively characterize the effects of PTN deficiency on hepatic lipid profiles in a model of high fat diet (HFD)-induced obesity and sex-related differences. Specifically, we sought to elucidate how PTN deficiency influences the hepatic lipidome and contributes to the establishment of insulin resistance-associated lipidomic signatures.

Materials and Methods

The experimental design incorporated wild-type (WT) and PTN knockout (KO) murine models, divided into six experimental groups (27 animals each), stratified by sex and genotype (male/female × WT/KO). Mice were subjected to three different dietary regimens over six months: continuous standard diet (STD-STD), high-fat diet for 3 months followed by standard diet for other 3 months (HFD-STD), and continuous high-fat diet (HFD-HFD).

After obtaining the liver homogenates, lipids were processed using all-in-one single phase extraction method with methanol:methyl tert-butyl ether (MeOH:MTBE) after addition of deuterated internal standards. Comprehensive lipid profiling was achieved by UHPLC-MS-QTOF employing both positive and negative electrospray ionization (ESI±) modes1. Data was processed with Agilent MassHunter Profinder 10.0, including normalization and signal drift correction2 performed with Matlab. Features in the quality control (QC) samples with coefficients of variation in their signals exceeding 20% were excluded. Lipid species were annotated using MS and MS/MS information with an in-house database1 and complementary lipid annotation tools. Finally, univariate and multivariate statistical analysis was performed.

Results

The analytical workflow successfully annotated approximately 420 lipid species, representing 24 different lipid subclasses, revealing significant differences across experimental groups.

Sex-based comparisons unveiled discriminatory features, highlighting phosphatidylcholines (PC), sphingomyelins (SM), ceramides (Cer), fatty acids (FA), and phosphatidylinositols (PI) species, indicating sex-specific differences in hepatic lipid metabolism.

Genotype-based comparisons revealed that KO mice had significant changes in FA and triacylglycerols (TG), suggesting PTN's role in regulating lipid metabolism and storage, possibly by affecting lipogenesis, fatty acid oxidation, or lipid uptake.

Dietary intervention comparisons demonstrated that the HFD-HFD group distinctly separated from both HFD-STD and STD-STD groups. The most significantly altered lipid classes included PC, phosphatidylethanolamines (PE), SM, TG, and FA species. Most lipid species were elevated in the HFD-HFD group, suggesting enhanced lipid uptake and subsequent metabolic alterations characterized by increased lipid synthesis, FA and TG accumulation, and the generation of potentially cytotoxic lipid species such as sphingomyelins.

Conclusions

This investigation revealed substantial lipidomic alterations in the murine liver, with distinct patterns influenced by sex, genotype, and dietary intervention. Notably, PC, SM, Cer, FA, and PI species were shown elevated in female subjects compared to males, while PTN knockout animals exhibited increased levels of FA and TG families relative to wild-type controls. Furthermore, the HFD-HFD group displayed the most distinctive lipidomic profile compared to other dietary intervention groups, with the greatest number of discriminatory features identified.

These findings underscore the utility of thorough lipidomics approaches in unravelling metabolic alterations associated with insulin resistance and provide a basis for future research to better understand the biological pathways underlying insulin resistance, energy metabolism, and liver fat accumulation.

References

[1] Martínez S, et al. (2024) J Lipid Res, 65(11):100671. PMID: 39395790
[2] Kuligowski J, et al (2015) Analyst 140(22):7810-7817. PMID: 26462549

Integrated multi-omics analysis of barley responses to combined drought and Fusarium pseudograminearum infection

Mikołaj Małecki (1), Dawid Perlikowski (2), Paweł Bednarek (1), Anna Piasecka (1)

(1) Institute of Bioorganic Chemistry PAS, (2) Institute of Plant Genetics PAS

Type of abstract: POSTER

Introduction and Aims:

Climate change caused an increase in the frequency and severity of drought events, which not only reduce cereal yields but also promote the spread of drought-adapted pathogens such as Fusarium pseudograminearum (Fp), the causal agent of Fusarium crown rot (FCR) (Liu and Liu, 2016). This project aims to elucidate the molecular trade-offs in barley (Hordeum vulgare L.) responses to simultaneous biotic and abiotic stresses. Two cultivars with contrasting drought tolerance are subjected to drought, Fp infection, and their combination under controlled conditions.

Material and Methods:

The experiment was conducted on two barley (Hordeum vulgare L.) cultivars differing in drought tolerance, grown under controlled greenhouse conditions. Plants were subjected to three stress treatments: drought, Fusarium pseudograminearum CBS109956 (Fp) infection, and combined drought and infection. Physiological parameters, including photosynthetic activity and transpiration rates, were monitored what helps in precise determination of infection initiation moment under drought. Metabolic profiling was performed using LC-MS coupled with ion mobility separation. Transcriptomic analyses were performed to assess the expression of stress-responsive marker genes, including Pathogenesis Related Protein 1 (PR1) and Dehydrin4, associated with drought adaptation and pathogen defense.

Results:

Preliminary LC-MS with ion mobility analyses indicate that all stresses induced reprogramming in the metabolome and lipidome of both barley genotypes. These changes are largely treatment-specific showing that combined stress is sense by plant as separate trigger. Importantly, most of metabolites and lipids in combined stress exhibited increased accumulation compared to the control conditions, which may indicate increased activation of metabolic pathways in response to the coexistence of abiotic and biotic stresses.

Physiological monitoring supported the omics findings, including reductions in photosynthesis and transpiration. The analysis of marker gene expression associated with drought and pathogen infection revealed that combined stress conditions significantly modulated transcriptional activity. In most instances, expression levels were reduced under dual stress compared to responses elicited by individual stress factors. This effect was particularly pronounced for the Pathogenesis-Related Protein 1 gene (PR1), suggesting that, under concurrent stresses, defensive resource allocation may be shifted toward drought-related tolerance mechanisms.

Conclusions:

Initial metabolite annotation, based on literature data and fragmentation spectra, revealed several compounds exhibiting stress-specific accumulation patterns. Under combined stress conditions, these included flavonoids, indole metabolites, and terpenoids. Among them feruloylagmatine, efficient antioxidant and tricine-methylated flavone derivatives, were characterized. An interesting group of compounds exhibited almost exclusive accumulation under combined stress conditions, with retention time profiles suggesting the presence of multiple isomers of flavonoids and hydroxycinnamic acid derivatives. Several glycero- and phospholipids were detected such us sphingomyelin, phosphatidylcholine or 2-acyl-sn-glycero-3-phosphocholines.

The integrated multi-omics approach — combining physiological, transcriptomics, proteomics, metabolomics, lipidomics, and phytohormone profiling — will map stress-specific and shared molecular signatures, identify regulatory hubs, and reveal factors facilitating Fp proliferation under water deficit. These findings will feed into a functional model of combined stress responses in cereals, providing potential biomarkers and molecular targets for breeding climate-resilient varieties.

References

[1] Liu X, Liu C. (2016) Effects of Drought-Stress on Fusarium Crown Rot Development in Barley. PLoS One. 11:e0167304. doi:10.1371/journal.pone.0167304

The synthetic TRPML1 agonist ML-SA1 reduces intracellular lipid accumulation by promoting the release of extracellular microvesicles via increased ceramide synthesis de novo

Rebeca Busto (1), Irene Chavarría-Cubel (1), Amanda Toledano-Martín (1), Yessenia L Molina (1), Milagros Lerma (1), Tamara Villanueva-Blanco (1), Javier Martínez-Botas (1), Diego Gómez-Coronado (1), Alberto Alcázar (1), Óscar Pastor (2)

(1) Servicio de Bioquímica Investigación, Hospital Universitario Ramón y Cajal-IRYCIS, Madrid, Spain, (2) Servicio de Bioquímica Clínica, Hospital Universitario Ramón y Cajal-IRYCIS, Madrid, Spain

Type of abstract: POSTER

Introduction and Aims: Antipsychotic drugs disrupt lipid homeostasis by inhibiting cholesterol biosynthesis and impairing intracellular cholesterol trafficking, leading to lipid accumulation in endolysosomes (1). Transient receptor potential mucolipin 1 (TRPML1) cation channels on late endosomal/lysosomal (endolysosome) membranes form pores for lysosomal calcium (Ca^{2+}) efflux (2). The overexpression of TRPML1 or its activation by agonists promotes cellular lipid clearance *in vitro* (2). Extracellular microvesicles (EVs) are small (diameter, 40–150 nm) intraluminal vesicles derived from large multivesicular endosomes and are released from cells. EVs have the ability to remove unwanted materials from cells, which may offer a different route of disposing of waste and decrease the cargo of overloaded lysosomes. This work aimed to characterize the mechanism by which the TRPML1 agonist ML-SA1 reduces intracellular lipid accumulation, and its potential to relieve antipsychotic-induced endolysosomal lipid accumulation by enhancing the secretion of extracellular microvesicles (EVs).

Material and Methods: Human Huh7 hepatocellular carcinoma cells were used in this study. The cellular content of DiI-LDL (LDL labelled with a fluorescent fatty acid) was analyzed using immunofluorescence microscopy. Lipidomic measurements were performed by LC-MS/MS (https://clipidomics.com). EVs were isolated, quantified using a nanoparticle tracking analyzer and analyzed by western blot. Gene expression was measured by qRT-PCR.

Results: ML-SA1 increased the secretion of EVs, thus reducing lipid overload and lysosomal size in cells treated with antipsychotics. ML-SA1 increased ceramide and dihydroceramide levels via ceramide synthesis *de novo*, by upregulating the genes encoding the enzymes involved in this process. Treatment with the Ca^{2+} chelator BAPTA-AM prevented decreased intracellular lipid accumulation, increased ceramide levels and secretion of EVs, indicating that intracellular Ca^{2+} movement through TRPML1 mediated the effects of MLSA1.

Conclusions: These findings indicate that TRPML1 activation stimulates the release of EVs via Ca^{2+} mobilization and increased ceramide synthesis *de novo*. This allows lipids to be released from the cell and improves the accumulation of endolysosomal lipids induced by antipsychotics.

Funding: This work was funded by project RTI2018-098113-B-I00, PI21/01173, PI22/01381 and PI24/00423.

References

[1] Canfrán-Duque A et al. (2013) J. Lipid Res. 54:310-324. PMID: 2317577
[2] Shen D et al. (2012) Nat. Commun. 3:731. PMID: 22415822

Uncovering the Effects of Air Pollution on Lung Health Using Human Bronchial Epithelial Cells and Organoid Models

Jordi Puigdemasa (1), Esmeralda Ayala (1), Barend van Drooge (1), Carmen Bedia (1)

(1) Institute of Environmental Assessment and Water Research (IDAEA-CSIC)

Type of abstract: POSTER

Effects of Air Pollutants on Skin Health: From Keratinocyte Toxicity to Lipidomic Remodeling in Reconstructed Skin

Carmen Bedia, Jordi Puigdemasa (1), Esmeralda Ayala (1), Virginie Garcia (2), Nathalie Andrieu-Abadie (2), Barend van Drooge (1)

(1) Institute of Environmental Assessment and Water Research (IDAEA-CSIC), (2) INSERM UMR1037. The Cancer Research Center of Toulouse (CRCT)

Type of abstract: POSTER

Lipidomic profiling to monitor phytoceramide biosynthesis in engineered Saccharomyces cerevisiae

Jordi Guixeras (1), Margalida Artigues Cladera (2), Magda Faijes (1), Antoni Planas (1)

(1) IQS, (2) Institut Químic de Sarrià (IQS)

Type of abstract: POSTER

Sphingolipids are amphipathic membrane components in eukaryotes that maintain architecture, regulate permeability, and provide rigidity. Their unique structure enables lipid raft formation, facilitating compartmentalized cellular signalling.
Phytoceramides, a ceramide subclass with phytosphingosine, are key for maintaining the skin barrier and preventing transepidermal water loss. They are among the most abundant ceramides in human skin, but levels decline with aging or conditions like atopic dermatitis. A low Cer[NP]/Cer[NS] ratio is linked to impaired barrier function, while a high ratio reflects healthy skin. Moreover, glycoconjugates of phytoceramides contribute to immune modulation, reducing pro-inflammatory cytokines, enhancing gut barrier integrity, and supporting microbiome stability under stress. Thus, its production has attracted increasing attention in pharmaceutical and cosmetic applications.

Despite its interest, phytoceramide availability is restricted by the limitations of natural extraction and the complexity of chemical synthesis. Metabolic engineering in Saccharomyces cerevisiae, a model organism with a well-characterized sphingolipid pathway, offers an attractive strategy to increase its production. Yeast S. cerevisiae naturally synthesizes several ceramide species, including dihydroceramide, phytoceramide, and their hydroxylated derivatives. Through targeted genetic modifications, such as overexpression or gene deletion, we designed yeast strains aimed at enhancing phytoceramide biosynthesis and accumulation.

To assess the efficiency of these engineering approaches, we implemented a lipidomic workflow based on LC-HRMS. This strategy enabled the identification and quantification of diverse ceramide species, providing a robust quality control for the metabolic design. The results highlight the utility of lipidomics not only to validate metabolic interventions but also to gain insights into the global lipid remodeling occurring in engineered yeast strains.

References

[1] Kuo, A. & Hla, T. Nat Rev Mol Cell Biol 25, 802 (2024)
[2] Yokose, U. et al. BMC Dermatol 20, 6 (2020)
[3] Zhang T et al., Front. Immunol. 10 (2019) 90
[4] Bendelac A et al., Annu Rev Immunol 25 (2007) 297-336
[5] Carreño LJ et al., Clin Transl Immunology 5 (2016) 4
[6] Wieland Brown LC et al., PLoS Biol. 7 (2013) 11

Evaluation of Tribrid Orbitrap Mass Spectrometer for Structural Lipidomics of Primary Cilia

Manal Farhat (1), David Da Silva (2), Cyril Colas (2), Thibaut Eguether (3), Emilie Destandau (2)

(1) Institute of Organic and Analytical Chemistry (ICOA), INSERM, Orléans, France, (2) Institute of Organic and Analytical Chemistry (ICOA), Orléans, France, (3) Laboratoire Interdisciplinaire pour l'Innovation et la Recherche en Santé d'Orléans Equipe thérapies innovantes ART ARNm INSERM US55

Type of abstract: POSTER

Introduction and Aims:

Primary cilia are antenna-like cellular structures critical for developmental signaling, yet their lipid composition remains largely unexplored (1). Lipidomics analyses are classically performed using TOF, QTOF, and triple quadrupole (TQ) mass spectrometers, providing low to moderate resolution and regularly coupled with ESI ionization (2). The goal of our study is to evaluate the contribution of ultra-high resolution Tribrid Orbitrap mass spectrometry, combined with diverse ionization sources, and to assess the added value of combining multiple fragmentation techniques on targeted lipid standards.

Materials and Methods:

Lipid profiling was performed using a Tribrid Orbitrap mass spectrometer (Thermo Fisher Scientific), operating in multiple ionization modes (ESI, APCI, SICRIT®). A mixture of eight lipid standards, including phosphatidylcholine, phosphatidylethanolamine, ceramides, and sphingomyelins, each at 10 µg/mL and covering all major lipid families, was used to evaluate the analytical workflow. Flow injection analysis (FIA) was performed combining HCD, CID, and UVPD fragmentation at various energies across different MS levels.

Results:

Full MS spectra confirmed the ionization of all standards, with detection of $[M+H]^+$, $[M+Na]^+$ and $[M-H]^-$ ions. FIA combining HCD, CID, and UVPD enabled fragmentation at expected sites across diverse lipid classes, improving and maximizing structural characterization and elucidation of closely related lipid species.

Conclusions:

The developed methods demonstrate efficient lipid fragmentation using a high-resolution tribrid Orbitrap system. Combining this approach with optimized separative conditions will enable a robust and reproducible LC-MS workflow for high-resolution, untargeted lipidomic characterization of primary cilia, supporting future research in this domain.

References

[1]) Gopalakrishnan J, Feistel K, Friedrich BM, et al. Emerging principles of primary cilia dynamics in controlling tissue organization and function. EMBO J. 2023;42(21):e113891. doi:10.15252/embj.2023113891

[2] Cajka T, Fiehn O. Comprehensive analysis of lipids in biological systems by liquid chromatography-mass spectrometry. Trends Analyt Chem. 2014;61:192-206. doi:10.1016/j.trac.2014.04.017

Uncovering Lipid Signatures of Inflammatory Cholestasis through RP-LC-MS

Sara Moreno Talavera (1), Carolina González Riaño, Jesús Fernández-Felipe (2), Francisco Javier Cuesto (2), Ángeles López-Gonzálvez (1), Francisco Javier Rupérez (1)

(1) UNIVERSIDAD SAN PABLO CEU, (2) La Paz University Hospital, Spain

Type of abstract: POSTER

Metabolomic and Lipidomic Profiling Identifies Novel Plasma Signatures in Patients with Transthyretin Cardiac Amyloidosis and Aortic Valve Stenosis Diseases

Ángela Peralbo-Molina (1), Gabriel Blanco-López (2), Emilio Blanco-López (3), Jesús Piqueras-Flores (4), Laura Mourino-Alvarez (2), María G. Barderas (2), Eduardo Chicano-Gálvez (1)

(1) Mass Spectrometry and Molecular Imaging Unit (IMSMI), Maimonides Biomedical Research, Institute of Cordoba (IMIBIC), Reina Sofia University Hospital, University of Cordoba, Spain, (2) Department of Vascular Physiopathology, Hospital Nacional de Parapléjicos, SESCAM, 45071 Toledo, Spain; Department of Vascular Physiopathology, Hospital Nacional de Parapléjicos, IDISCAM, (3) Department of Vascular Physiopathology, Hospital Nacional de Parapléjicos, IDISCAM; Department of Cardiology, Ciudad Real General University Hospital, 13005 Ciudad Real, Spain, (4) Heart Failure and Inherited Cardiovascular Diseases Unit, Ciudad Real General University Hospital, 13005 Ciudad Real, Spain; Faculty of Medicine, University of Castilla-La Mancha, 13071 Ciudad Real, Spain

Type of abstract: POSTER

Introduction and Aims:

Transthyretin cardiac amyloidosis and aortic valve stenosis are cardiovascular diseases which involve complex metabolic dysregulation. To explore the biochemical signatures underlying these disorders, we carried out a metabolomic and lipidomic study in plasma from patients with amyloidosis and aortic valve stenosis diseases, compared to patients with stenosis, both without type 2 diabetes. The aim was to identify novel lipid and metabolite biomarkers associated with amyloid pathology.

Material and Methods:

Patients were recruited at the Cardiac Surgery unit of the Hospital Universitario de Toledo (Toledo, Spain) and at Department of Cardiology, Ciudad Real General University Hospital.

Plasma samples underwent comprehensive metabolomic and lipidomic profiling using high-resolution mass spectrometry. Statistical evaluation included PLS-DA, t-test, volcano plots, and fold-change analysis to identify significantly different compounds.

Results:

The analysis revealed alterations across multiple lipid classes. Significant differences were observed in triglyceride TG 16:1/18:1/24:1, phosphatidylethanolamines (PE 36:2/20:4, PE O-38:8, PE 18:0/22:6, PE 18:0/22:4), phosphatidylcholines (PC 18:5/24:5, PC 9:0/34:9, PC 40:8, PC O-46:5), phosphatidylinositol (PI 36:2), N-acyl-lysophosphatidylethanolamin (LNAPE 20:4/N-19:0), and the sphingolipid trihexosylceramide. Additionally, octanoylcarnitine (acylcarnitine C8) was significantly modulated. These findings point to disruptions in membrane lipid remodeling, fatty acid metabolism, and mitochondrial function.

Conclusions:

This study demonstrates that patients with amyloidosis and stenosis present a distinct metabolomic and lipidomic plasma signature compared to stenosis. Identified lipid species may provide novel biomarkers and enhance understanding of amyloid-associated cardiac dysfunction.

References

[1] Geng C, et al. (2025) Frontiers in Immunology, 16:1509773. PMID: 40074840
[2] Bernhard OK, et al. (2021) Molecular Omics, 17(7):636-645. PMID: 34319177

Plasma lipidomics signature reveals differential glucose use in idiopathic thoracic aortic aneurysm in patients with tricuspid and bicuspid aortic valves

Ariadna Martin-Blazquez (1), Caridad Díaz (2), María deSancha-Maluenda (1), Rocio Ruiz-Jimenez (1), Patricia Mena Garcia (2), Alicia Donado (3), Angeles Heredero (3), Maria Luisa Garcia-Gil (4), Gonzalo Aldamiz-Echevarria (3), Marta Martin-Lorenzo (1), Gloria Alvarez-Llamas (1)

(1) Instituto de Investigación Sanitaria Fundación Jiménez Díaz (IIS-FJD, UAM), Madrid, Spain, (2) Fundación MEDINA, Granada, Spain, (3) Hospital Universitario Fundación Jiménez Díaz-UAM, Madrid, Spain, (4) Centro Nacional de Microscopia Electrónica (ICTS-CNME). Universidad Complutense de Madrid, Madrid, Spain

Type of abstract: POSTER

Introduction and Aims:

Thoracic aortic aneurysm (TAA) develops asymptomatically with potentially fatal consequences and its diagnosis is often incidental. The lack of knowledge in pathophysiological mechanisms and markers of aortic dilation lead to inexistent drug therapies and early diagnosis. TAA is mostly idiopathic, although its prevalence is significantly higher in subjects with bicuspid aortic valve (BAV) for unknown reasons. Our hypothesis is that there is a reflection in plasma of the mechanisms involved in the development of TAA, which differ depending on whether the aortic dilatation is associated with a BAV or a tricuspid aortic valve (TAV). Our objective is to identify a plasma fingerprint of TAA, for both BAV and TAV patients, and to decipher their particular pathophysiological mechanisms.

Material and Methods:

Samples were collected from 110 patients and classified in TAA or control (C) if no aortic dilatation was present. Patient subgroups were done according to the aortic valve type (BAV or TAV). Untargeted LC-MS/MS was carried out by reversed phase and hydrophilic interaction chromatography in positive and negative electrospray ionization modes (Plasma, TAV-C, n=6; TAV-TAA, n=6; BAV-C, n=8; BAV-TAA, n=7). Identification was achieved by NIST and HMDB databases and MS/MS spectra. Metabolites of interest (FDR-adjusted p-value≤0.05) were validated using commercially available standards by LC-MS/MS in selected reaction monitoring mode (SRM) in a different patient cohort (Plasma, TAV-C, n=22; TAV-TAA, n=12; BAV-C, n=34; BAV-TAA, n=30) (t-test/Mann-Whitney, p-value≤0.05). Relevant metabolism alterations found were confirmed directly in aortic tissue (TAV-C, n=12; TAV-TAA, n=14; BAV-C, n=13; and BAV-TAA, n=11).

Results:

We found increased plasma levels of acetylcarnitine (p-value = 0.0261), glutarylcarnitine (p-value = 0.0114) and myristoylcarnitine (p-value = 0.0033) in TAV patients. Myristoylcarnitine (AUC = 0.833) and glutarylcarnitine (AUC = 0.792) demonstrated the strongest performance in distinguishing between C and TAA patients and myristoylcarnitine significantly correlated with ascending aortic diameter (p-value = 0.0074). This was accompanied by an in-situ shift of glucose towards hexosamine biosynthetic pathway; overexpression of GFTP2 (p-value= 0.0487) and UAP1 (p-value = 0.0086) and an increase of glycosaminoglycan pools in the aortic media (p-value = 0.0026). Contrastingly, the BAV cohort showed increased plasma levels of LPC 16:0 (p-value = 0.0321) and LPE 18:0 (p-value = 0.0260) in TAA patients together with an in-situ glucose shift to maintain redox homeostasis through the pentose phosphate pathway, evidenced by increased G6PDH activity (p-value = 0.0147) and decrease in NADP+/NADPH ratio (p-value = 0.0040).

Conclusions:

The idiopathic development of TAA driven by glucose metabolic flexibility is reflected differently in the plasma of patients with BAV and TAV. This supports an individualized diagnosis based on the valve type and highlights the need for personalized therapeutic approaches in these two patient groups.

Deep lipidome coverage with high confidence annotations using Orbitrap Astral Zoom MS

Susan Bird (1)

(1) Thermo Fisher Scientific

Type of abstract: POSTER

Searching for FAHFA transporters in blood

Dovile Milonaityte (1)

(1) Institute of Physiology, Czech Academy of Sciences

Type of abstract: POSTER

Adipose tissue is the source of ketone bodies in advanced heart failure: direct analysis of fat metabolic remodeling

Michaela Hradecna (1)

(1) Institute of Physiology of the Czech Academy of Sciences

Type of abstract: POSTER

Effect of 6-month Nutritional and Lifestyle Intervention on Oxylipines and Inflammatory Markers in MASLD patients.

Maria Magdalena Quetglas Llabrés (1), Margalida Monserrat-Mesquida (1), Cristina Bouzas (1), Silvia García (1), David Mateos, Miguel Casares, Lucía Ugarriza, Isabel Llompart, Antoni Sureda, Antoni Pons (1), Josep A. Tur

(1) Universitat de les Illes Balears

Type of abstract: POSTER

Introduction: Metabolic dysfunction-associated steatosis liver disease (MASLD) is the most common chronic liver disease worldwide and is closely linked to atherogenic dyslipidemia. Although lifestyle interventions can improve cardiometabolic health, the specific relationship between intrahepatic fat content (IFC) and oxilipines levels remains poorly characterized.

Methods: Forty patients with MASLD completed a 6-month lifestyle intervention based on a Mediterranean diet pattern. Participants were stratified into two groups according to their IFC reduction: “reduce more” (n=20) and “reduce less” (n=20). Anthropometric, clinical, dietary, and physical activity parameters were assessed, along with plasma levels of oxylipins (measured by GC-MS) and inflammatory markers. Repeated-measures ANCOVAs (adjusted for age and sex) were performed to evaluate time and group effects. Pearson correlation analyses explored associations between circulating levels of oxylipins and interleukins, as well as between IFC and both lipid mediators and inflammatory markers.

Results: All participants showed improvements in anthropometric parameters after the intervention. However, only those with greater IFC reductions showed significant improvements in clinical markers, including glucose, HbA1c, triglycerides, and liver enzymes. This group also reduced their energy intake and consumed less saturated and trans fatty acids. Greater IFC reductions were associated with significant decreases in specific oxylipins, particularly 17-hydroxy-docosahexaenoic acid (17DoHE) and maresin-1 (MaR1). Correlation analyses revealed a positive association between prostaglandin F2α (PGF2α) and interleukin-1 receptor antagonist (IL-1ra). Moreover, IFC was positively correlated with several lipid mediators (15-hydroxyeicosatetraenoic acid (15HETE), 17DoHE, 3-hydroxytetradecanoic acid (3HMYR), 12-hydroxyoctadecanoic acid (12HEST), lipoxin B4 (LXB4), and PGF2α) and inflammatory markers (IL-1ra, tumor necrosis factor alpha (TNFα), and leptin), supporting the role of oxylipins in hepatic fat accumulation and inflammation.

Conclusion: A 6-month Mediterranean-based lifestyle intervention significantly improved metabolic health and reduced IFC in patients with MASLD. These reductions were associated with favorable changes in oxylipin and inflammatory profiles, highlighting the potential of oxylipins as biomarkers of liver fat modulation and inflammatory status.

Targeted lipidomics reveals systemic effects of pectin intervention in LTP-allergic patients.

Jaime Morillas (1), Andrea Macías-Camero (1), Francisca Gómez (2), Natalia Pérez-Sánchez (2), Domingo Barber (1), Coral Barbas (3), Cristobalina Mayorga (4), María José Torres (2), Craig Wheelock (5), Antonio Checa (5), Alma Villaseñor (1)

(1) Instituto de Medicina Molecular Aplicada Nemesio Díez (IMMA-ND), Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Universidad San Pablo-CEU, CEU Universities, Boadilla del Monte, Spain., (2) Allergy Clinical Unit, Hospital Regional Universitario de Málaga, Málaga, Spain., (3) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Boadilla del Monte, Spain., (4) Allergy Clinical Unit, Hospital Regional Universitario de Málaga, Málaga, Spain. Allergy Research Group, Instituto de Investigación Biomédica de Málaga (IBIMA), Málaga, Spain., (5) Unit of Integrative Metabolomics, Institute of Environmental Medicine, Karolinska Institute, Stockholm, Sweden.

Type of abstract: POSTER

Introduction and Aims:

Lipid mediators play a crucial role regulating immune and inflammatory responses, and their dysregulation has been linked to the development of food allergy (FA). Among these mediators, oxylipins act as signaling molecules involved in the balance of inflammatory pathways, whereas sphingolipids regulate epithelial barrier integrity and immune homeostasis.

In the Mediterranean area, lipid transfer protein (LTP) allergy represents the most prevalent form of FA[1]. Novel dietary strategies such as pectin interventions have emerged as promising approaches for FA, modulating the gut microbiome. However, the mechanisms underlying these effects remain poorly understood.

To address this gap, we have implemented a targeted metabolomics strategy including three complementary panels: microbial-derived metabolites (short chain fatty acids and bile acids), lipid mediators (oxylipins and sphingolipids) and polar metabolites (polar lipids, organic nitrogen compounds and organic acids). While all panels provided valuable insights, in the present work we focus on the lipidomics results, given the central role of lipid mediators in immune regulation.

The aim of this study was to evaluate systemic lipid mediator dynamics in LTP-allergic patients undergoing a pectin-based intervention, within the framework of a broader targeted metabolomics approach.

Materials and Methods:

34 LTP-allergic patients were enrolled in a double-blind, placebo-controlled food challenge (DBPCFC) and randomized to receive either placebo (n = 9), a citrus-derived pectin with low degree of esterification (CP-DElow, n = 13), or an apple-derived pectin with high degree of esterification (AP-DEhigh, n = 12), administered twice daily for two months. Serum samples were collected at baseline (T1) and after intervention (T2) and immediately stored at –80 °C until analysis.

Sample analysis was performed at Karolinska Institutet using liquid chromatography coupled to triple quadrupole mass spectrometry (LC-QqQ-MS). Free oxylipins and sphingolipids were analyzed in independent worklists using panel-specific methodologies. For oxylipins, sample preparation included solid-phase extraction (SPE) in a 96-well plate, whereas sphingolipids were extracted using methanol:water (MeOH:H_2O). Oxylipins were measured on a Waters Xevo® TQ-XS, and sphingolipids on a Waters Xevo® TQ-S. Data were acquired in negative and positive ionization modes, respectively, using dynamic multiple reaction monitoring (dMRM).

Signal integration and data processing were performed using MassLynx® and TargetLynx® software (Waters Corporation). Oxylipins were quantified by absolute quantification using calibration curves with internal standards, whereas sphingolipids were analyzed by relative quantification based on peak area integration.

Results:

Initial detection included a larger set of lipid mediators; after QA/QC procedures (TUS clustering and %CV filtering in QC samples), 169 lipid mediators (78 oxylipins and 91 sphingolipids) were included in the final analysis. Group comparisons and tolerance-related analysis were performed using fold change (FC), which revealed distinct lipid mediator shifts associated with the pectin intervention.

Univariate statistical analyses were conducted using non-parametric tests. Differences among the three intervention groups (placebo, CP-DElow, and AP-DEhigh) were assessed with the Kruskal–Wallis test, whereas comparisons between tolerant and non-tolerant patients were performed using the Mann–Whitney U test.

Oxylipins and sphingolipids exhibited distinct patterns across both analyses. In the comparison between treatment groups, the largest differences were observed between CP-DElow and AP-DEhigh, with representative changes in CerP(d18:1/18:0) and LacCer(d18:1/18:1). Regarding tolerance, oxylipin alterations such as 12,13-DiHOME were detected within the CP-DElow group, whereas sphingolipid differences, such as SM(d18:1/18:1), were observed in both pectin interventions.

Conclusion:

In summary, this study confirmed that targeted metabolomics is a powerful approach to investigate systemic effects of pectin interventions in LTP-allergic patients. Distinct oxylipin and sphingolipid profiles differentiated between the two pectin treatments and were further associated with clinical tolerance, highlighting the potential of lipidomics for translational applications.

References

[1] (1) Gomez F, et al. (2025) Carbohydrate Polymers, 369. DOI: 10.1016/j.carbpol.2025.124236

Lipidomic Profiling Reveals Altered Fatty Acid and Carnitine Metabolism in Cofactor-Induced LTP Anaphylaxis

Blanca Raposo Martínez (1), Rocío Casas-Saucedo (2), María Isabel Delgado-Dolset (3), René Neuhaus (4), Patricia Mir-Ihara (5), María Escribese (6), Rosa Muñoz Cano (7), Alma Villaseñor (8)

(1) Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Instituto de Medicina Molecular Aplicada – Nemesio Díez (IMMA-ND), Universidad San Pablo-CEU. Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe, (2) Unidad de Alergología. Fundació Privada Asil Granollers. Hospital de Granollers, (3) Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Instituto de Medicina Molecular Aplicada – Nemesio Díez (IMMA-ND), Universidad San Pablo-CEU, (4) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities., (5) Servicio de Alergología. Hospital Clínic. Universidad de Barcelona. METRI2A-Instituto de Investigación August Pi i Sunyer., (6) Departamento de Ciencias Médicas Básicas, Facultad de Medicina, Instituto de Medicina Molecular Aplicada – Nemesio Díez (IMMA-ND), (7) Servicio de Alergología. Hospital Clínic. Universidad de Barcelona. METRI2A-Instituto de Investigación August Pi i Sunyer, (8) Centro de Metabolómica y Bioanálisis (CEMBIO), Facultad de Farmacia, Universidad San Pablo-CEU, CEU Universities, Urbanización Montepríncipe

Type of abstract: POSTER

Introduction and Aims

Food allergies, affecting approximately 13% of the population in Europe, is a significant public health concern. In southern Europe, lipid transfer protein (LTP) allergy is the most common primary food allergy, with clinical manifestations ranging from mild urticaria to potentially life-threatening anaphylactic reactions. Since LTPs are present in a wide variety of plant foods, affected individuals often experience reactions to multiple allergens. Cofactors can enhance allergic responses or trigger anaphylaxis, with some individuals only experiencing reactions when cofactors are present. Recently, the potential involvement of lipids in allergy has been proposed, however, their role in anaphylaxis is still largely unknown. This study aims to investigate the mechanisms behind the cofactor effect in LTP anaphylaxis by assessing whether aspirin/exercise induce a distinct lipidomic signature in fatty acids and carnitines even in the absence of allergen exposure.

Material and Methods

Four adult groups were recruited: healthy controls (A) (n=6), atopic controls (with asthma/rhinitis but no food allergy) (B) (n=6), LTP-sensitized individuals with no food allergy (C) (n=5) and cofactor-induced LTP anaphylaxis (D) (n=9). Individuals participated in two hospital visits. At visit 1, patients underwent an exercise test and at visit 2, patients took 500 mg of aspirin. Serum samples were taken before the cofactor exposure (T0) and at 2 points post-treatment/ intervention (30min for exercise, 1h for aspirin, and 2h for both).

Untargeted lipidomic profiling was carried out using liquid chromatography coupled to quadrupole time-of-flight mass spectrometry (LC-QTOF-MS, Agilent 6545). Samples from the exercise and aspirin intervention were analyzed in two batches in both positive and negative ionization modes (ESI+ and ESI−). A pooled quality control (QC) sample was injected throughout each batch to monitor analytical stability and reproducibility. Lipid annotation was performed using a highly reliable in-house lipid database *(1)* in Profinder. Data were normalized using the Total Useful Signal (TUS) approach, and data quality was assessed by unsupervised principal component analysis (PCA). Group comparisons were subsequently performed using the Mann-Whitney U test for univariate statistics for each time point.

Results

This method allowed the annotation of 29 fatty acids and 18 acylcarnitines. PCA model demonstrated clustering of quality control (QC) samples in both aspirin and exercise experiments after normalization of the raw data, confirming analytical reproducibility and data quality. The anaphylaxis group (D) showed distinct lipid changes compared to healthy controls (A), including some fatty acids and acylcarnitines, independent of cofactor exposure. Before taking aspirin, the group D, already had a distinct profile of these lipids at baseline compared to group A, which remained decreased over time. These changes although not significant followed the same trend at baseline before exercise. Additional lipid changes including arachidonic

(AA) and docosahexaenoic acids (DHA), were observed at 30 minutes after exercise between groups A and D which seem to diminish after 2h.

Conclusions

These alterations suggest an intrinsically different lipid phenotype in the group of cofactor-induced LTP anaphylaxis compared to the healthy controls which appears unaffected by aspirin intake. In contrast, exercise leads to a different lipidomic profile between the two groups. Further analyses must be done to understand the mechanisms underlying cofactor-induced anaphylaxis.

References

[1] Martínez S, et al. (2024) J Lipid Res, 65(11):100671. PMID: 39395790

Increased serum bile acid concentrations in obese patients after bariatric surgery.

Oliwia Lange (1), Aleksandra Budny (2), Agata Janczy (2), Maciej Wilczynski (2), Michal Szymanski (2), Monika Proczko-Stepaniak (2), Adriana Mika (2), Tomasz Sledzinski (2)

(1) University of Gdansk, (2) Medical University of Gdansk

Type of abstract: POSTER

Obesity is currently a serious health and social problem, and for individuals with a BMI exceeding 35 bariatric surgery is an effective treatment. Bile acids (BA) in the blood play a regulatory role, influencing, among other things, insulin sensitivity. The aim of this study was to examine the concentrations of 18 different bile acids in the serum of obese patients during preparation for surgery (low-calorie diet), immediately before the procedure, and after the procedure.
Blood was collected from 25 patients before the low-calorie diet, during the diet, immediately before laparoscopic sleeve gastrectomy (LSG), and 3, 6, 9, and 12 months after the procedure. BA concentrations were measured using liquid chromatography-mass spectrometry (LC-MS).

Our study showed that during the low-calorie diet, a slight decrease of BA concentrations in paients serum, while after LSG, serum BA concentrations increased for up to 12 months, that was associated with the improvement of insulin sensitivity. However, not all BA concentrations increased after the procedure. Six secondary BAs, which synthesis is influenced by intestinal bacteria, did not change.

The presented study allowed for a thorough assessment of many BAs at various stages of bariatric treatment. The increase in most BAs after LSG is a beneficial change that likely contributes to improved insulin sensitivity in these patients. However, the fact that the concentration of six secondary BAs did not change indicates that this is related to changes in the composition or metabolism of gut bacteria after LSG. These results highlight the need to examine the composition of gut bacteria during treatment of patients with obesity and suggest that the use of appropriate diet/probiotics may enhance the beneficial effects of bariatric treatment.

Investigating the Relationship Between Guselkumab treatment and Signaling Lipid Levels in blister and plasma samples from psoriasis patients

Marielle Pascalle van der Peet (1), Jannik Rousel (2), Menthe Bergmans (3), Firdaws Badmus (4), Tom Ederveen (4), Tessa van der Kolk (3), Pascal Maas (1), Alida Kindt (1), Amy Harms (1), Martijn van Doorn (5), Thomas Hankemeier (1), Robert Rissmann (3)

(1) Leiden university, (2) Centre for Human Drug research, (3) Centre for Human Drug Research, (4) Radboud University Nijmegen, (5) Erasmus Medical Centre

Type of abstract: POSTER

Introduction:
Psoriasis, a common chronic inflammatory skin disease, is characterized by erythematous, scaly plaques on the skin. These plaques result from excessive keratinocyte proliferation and immune-mediated inflammation. Once considered solely a skin disorder, psoriasis is now recognized as a systemic immune-mediated condition driven by complex interactions between skin-resident cells and immune pathways.
A central driver of the disease pathogenesis is the IL-23/Th17 axis, where IL-23 sustains Th17 cells that secrete cytokines (IL-17, IL-22), thereby promoting keratinocyte activation and inflammation[1]. Guselkumab, a selective IL-23 inhibitor, has demonstrated clinical efficacy, reflected by improved Psoriasis Area and Severity Index(PASI) scores. Yet, broader systemic and local molecular effects of IL-23 blockade remain incompletely understood.

Method:
A randomized, double-blind, controlled study was conducted in 26 psoriasis patients with mild (PASI < 5) to moderate-to-severe disease (PASI <10). Of these, 20 received guselkumab and 6 received a placebo. Samples were collected at baseline, after 28 days, and after 112 days of treatment. In patients, plasma samples along with suction blisters were taken from unaffected non-lesional skin and peri-lesional skin (next to the lesions), while only plasma and non-lesional blister fluid samples were collected from healthy volunteers. All samples were measured using a targeted HPLC-MS method covering 250 targets, including fatty acids, bile acids, oxylipins, endocannabinoids, and lysophospholipids[2].

Results and discussion:
A total of 114 metabolites were identified in non-lesional and peri-lesional blister fluid, and 127 metabolites in plasma. At baseline, significant metabolic differences were observed between patients and healthy volunteers, particularly in peri-lesional blister fluid compared to plasma, underscoring the relevance of local skin metabolism in psoriasis pathophysiology. Post-treatment, the differences between patients and healthy volunteers notably reduced in blister fluid but less consistently in plasma, suggesting a stronger impact of guselkumab on the local biochemical environment.

Key findings included elevated sphingosine species in perilesional blisters pre-treatment and decreased post-treatment, consistent with reduced keratinocyte activation upon IL-23 suppression. Polyunsaturated fatty acids from the omega-3 and omega-6 families were downregulated in blister fluid. This aligns with previous serum-based observations, although the effect on plasma in our study was limited.

Oxylipins showed preferential metabolism of arachidonic acid via the lipoxygenase (LOX) pathway (13-HODE, 15-HETE, and 15-HETrE) rather than the cyclooxygenase (COX) pathway (PGE2, PGA2, and 8-iso-PGE1). This is in line with the known defective COX-2 induction in psoriatic fibroblasts, which persists despite. While LOX pathway activity declined with guselkumab, mirroring reduced Th17-driven inflammation. COX activity remained impaired despite increased arachidonic acid substrate availability, potentially indicating alternative metabolic adaptations.

At baseline, bile acid alterations were the most consistent across compartments. The secondary bile acid deoxycholic acid (DCA) was reduced at baseline but normalized in peri-lesional blisters after treatment and remained suppressed in plasma. As secondary bile acids are known to be decreased in psoriasis, these findings suggest compartment-specific recovery

during IL-23 blockade.

Conclusion:
These findings reveal distinct local and systemic metabolic changes in psoriasis, with guselkumab showing more pronounced effects in the skin than in systemic circulation. Targeted lipidomics in paired suction blister fluid and plasma samples provides valuable insights into disease mechanisms and potential metabolic biomarkers for monitoring therapeutic response. These findings advance understanding of psoriasis pathology and may guide future biomarker development and treatment strategies.

References

[1] G. Girolomoni et al., “The role of IL-23 and the IL-23/TH17 immune axis in the pathogenesis and treatment of psoriasis,” J. Eur. Acad. Dermatol. Venereol., vol. 31, no. 10, pp. 1616-1626, 2017, doi: 10.1111/jdv.14433.
[2] W. Yang, J. C. Schoeman, X. Di, L. Lamont, A. C. Harms, and T. Hankemeier, “A comprehensive UHPLC-MS/MS method for metabolomics profiling of signaling lipids: Markers of oxidative stress, immunity and inflammation,” Anal. Chim. Acta, vol. 1297, p. 342348, Apr. 2024, doi: 10.1016/j.aca.2024.342348.

A lipidomic exploration of the effects of high-intensity interval exercise in healthy men after metformin intake

René Neuhaus (1), Thomas Meikopoulos (2), Ana Gradillas (1), Carolina González-Riaño (1), Georgios Theodoridis (2), Stefanos Nikolaidis (3), Vassilis Mougios (3), Coral Barbas (1), Helen Gika (4), Alma Villaseñor (5)

(1) Centre of Metabolomics and Bioanalysis (CEMBIO), Faculty of Pharmacy, University San Pablo CEU, Madrid, Spain, (2) Department of Chemistry, Aristotle University of Thessaloniki & Biomic AUTh, Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece, (3) Laboratory of Evaluation of Human Biological Performance, School of Physical Education and Sport Science at Thessaloniki, Aristotle University of Thessaloniki, Greece, (4) Department of Medicine, Aristotle University of Thessaloniki & Biomic AUTh, Center for Interdisciplinary Research and Innovation (CIRI-AUTH), Thessaloniki, Greece, (5) Centre of Metabolomics and Bioanalysis (CEMBIO), Faculty of Pharmacy & Departamento de Ciencias Médicas Básicas, Instituto de Medicina Molecular Aplicada (IMMA) Nemesio Díez, Facultad de Medicina, Universidad San Pablo-CEU, Madrid, Spain

Type of abstract: POSTER

Introduction and Aims:

Physical activity is essential for good health, known to produce large changes in the concentrations of multiple metabolites, including lipids, and widely recommended to prevent and treat obesity-related complications such as type 2 diabetes mellitus (T2DM). Metformin is a first-line anti-hyperglycemic drug for T2DM treatment and was shown to also alter plasma lipid composition. In a recent study it was found that high-intensity interval exercise (HIIE) affected metformin pharmacokinetics compared to rest (1). In this scenario, changes in the plasma concentrations of individual lipids could play an important role in the response to HIIE under metformin intake.Thus, the aim of this work was to explore the dynamic changes in the plasma lipidome over a timeframe of several hours between HIIE and rest, both under metformin intake.

Material and Methods:

In this study, nine healthy male individuals participated in two sessions lasting 24h. In both sessions they took 1000mg of metformin in the beginning and afterwards performed either a HIIE test or rested. Blood samples were collected before taking metformin and during each session at 13 timepoints and plasma was obtained by centrifugation. A single-phase lipid extraction was performed using methanol and methyl-tert-butylether and samples were analyzed using an Agilent 1290 Infinity II UHPLC system coupled to an Agilent 6545 QTOF mass spectrometer. Data were acquired in both ionization modes in separate runs. Lipid annotation was achieved by using LipidAnnotator, MS-DIAL, a published in-house plasma lipid database (2) and manual inspection of fragmentation patterns from iterative-MS/MS runs. Paired Wilcoxon test was applied to compare each timepoint between the two sessions.

Results:

The annotation process yielded 247 lipids from 16 lipid classes. The statistical comparison between HIIE and rest at the different timepoints revealed dynamic changes of these lipids, mainly sphingomyelins, glycerophosphocholins, triacylglycerols, fatty acids and acyl carnitines. These changes were followed in time up to 12 h, also showing the effect of meals taken during the sessions. We hypothesize the changes are a synergistic effect of HIIE and metformin on the lipidome, with the effect of HIIE predominating.

Conclusions:

The observed changes provide crucial insights into understanding the dynamic and complex physiological response of humans to HIIE under an antidiabetic drug and pinpoint the main biological processes implicated in maintaining a healthy status.

References

[1] Nikolaidis S, et al. (2020) Scand. J. Med. Sci. Sports, 30 (5), 858−864. PMID: 31975547
[2] Martínez S, et al. (2024) J Lipid Res., 65(11):100671. PMID: 39395790

Feasibility of identifying gallbladder cancer by simple and direct analysis of human bile using paper spray ionization mass spectrometry

Sangwon Cha (1), Donghoon Lee (1), Eunjin Jang (2), Jihye Choi (1), Hoeil Chung (2)

(1) Dongguk University, (2) Hanyang University

Type of abstract: POSTER

Gallbladder (GB) cancer is the most aggressive malignancy of the biliary tract, and its diagnosis may benefit from analytical approaches targeting bile, a fluid in direct contact with the tumor. In this study, we developed a paper spray ionization mass spectrometry (PSI-MS) method for direct bile analysis with minimal pretreatment, aiming to identify metabolite signatures specific to GB cancer. Diluted bile samples (n=74) from healthy individuals and patients with gallstones, GB polyps, hepatocellular carcinoma (HCC), and GB cancer were analyzed by PSI-MS. The use of alum-treated PSI tips and alcohol-based spraying solvents efficiently generated bile metabolite fingerprints with reduced spectral complexity, even for these high-salinity bile samples. Initial PSI-MS screening revealed a distinct reduction in phospholipid-related signals (m/z 731–860) in the GB cancer group. Ratiometric analysis further identified a significantly elevated signal at m/z 732, corresponding to the protonated ion of phosphatidylcholine (PC) 32:1, and a decreased signal at m/z 758 ($[PC\ 34:2 + H]^+$) in GB cancer bile compared to other groups. These trends in GB cancer fingerprints were further validated by targeted selected reaction monitoring (SRM), confirming the enhanced PC 32:1 ratio in GB cancer bile. Our findings demonstrate that PSI-MS enables rapid, direct profiling of bile metabolites and holds strong potential as a minimally invasive clinical tool for GB cancer diagnosis.

Quantitation of Lipids via phosphorous using inductively coupled plasma mass spectrometry with oxygen reaction gas.

Anne M. Roberts (1), Shaima Nazzar (1), Blaine Roberts (1)

(1) Emory School of Medicine

Type of abstract: POSTER

Spatial Multi-Omics Reveal Lipid Biomarkers of Tumor Heterogeneity in Colorectal Cancer

Albert Maimó-Barceló (1), Ramón María Rodríguez (1), Karim Pérez-Romero (1), Cristina Huergo (2), Teresa Ximelis (1), José Andrés Fernández (2), Gwendolyn Barceló-Coblijn (1)

(1) Fundació Institut d'Investigació Sanitària Illes Balears (IdISBa), (2) Universidad del País Vasco

Type of abstract: POSTER

Dietary Radish Bioactives and Their Impact on Brain Lipidome Remodeling and Oxysterol Homeostasis in Obesity

Vladimiro Cardenia (1), Fabio Vivarelli (2), Silvia Cirillo, Moreno Paolini (2), Maria Teresa Rodriguez-Estrada, Donatella Canistro

(1) University of Turin, (2) University of Bologna

Type of abstract: POSTER

Introduction and Aim:

Obesity is a major public health concern, closely associated with oxidative stress, systemic inflammation, and neurodegenerative risk factors (1). High-fat diets can disrupt brain lipid homeostasis, leading to altered cholesterol metabolism and accumulation of toxic oxysterols such as 27-hydroxycholesterol (27-HC), implicated in Alzheimer's and Parkinson's diseases (2). Brassica vegetables, including Raphanus sativus cv. Sango sprouts, are rich in bioactive compounds such as isothiocyanates and anthocyanins, known for antioxidant and lipid-modulating properties. Based on prior evidence of Sango sprout juice (SSJ) mitigating redox imbalance and dyslipidemia in obese rats, the present study aimed to evaluate the effect of SSJ supplementation on brain lipid composition and cholesterol oxidation in a non-genetic model of diet-induced obesity.

Materials and Methods:

Thirty-five male Sprague Dawley rats were divided into regular diet (RD, 4.5% fat) and high-fat diet (HFD, 34% fat) groups. After 10 weeks, HFD-fed animals were subdivided into four groups: continued HFD, HFD switched to RD, HFD supplemented with SSJ (75 mg/kg/day), and HFD switched to RD with SSJ supplementation. SSJ was prepared by freeze-drying sprout juice. After a 28-day intervention, brains were collected, and lipid extraction performed using the Folch method. Fatty acid composition was determined via GC-FID, sterols and cholesterol oxidation products (COPs/oxysterols) via Fast-GC/MS, and statistical analysis applied (ANOVA, PCA).

Results:

HFD increased brain lipid infiltration compared to RD but did not significantly alter overall fatty acid composition, which remained predominantly unsaturated (~58%). Notably, linoleic acid (LA) content was halved under HFD. Brain cholesterol levels decreased with HFD compared to RD but increased significantly when switched back to RD, regardless of SSJ. The sterol profile was largely dominated by cholesterol (~99%). HFD markedly elevated cholesterol oxidation (total oxysterols 63.1 mg/g), particularly increasing 27-HC infiltration across the blood-brain barrier compared to RD (0.24 vs 0.13 mg/g). SSJ supplementation with HFD reduced oxysterol accumulation, with effects comparable to switching from HFD to RD, especially lowering 27-HC and α-epoxycholesterol. Although 24(S)-hydroxycholesterol showed only a non-significant increasing trend under HFD, PCA revealed that SSJ was inversely associated with 24(S)-HC and 27-HC, indicating a protective effect against cholesterol oxidation.

Conclusions:

The present study demonstrated that while SSJ did not influence overall brain lipid infiltration or fatty acid profile, it significantly counteracted cholesterol oxidation in rats fed a high-fat diet, particularly reducing neurotoxic oxysterols. These findings highlight the potential of Raphanus sativus Sango sprout juice as a dietary intervention to mitigate diet-induced brain oxidative imbalance and possibly lower the risk of neurodegenerative processes. Further studies are needed to clarify the mechanistic link between LA levels, cholesterol metabolism, and oxysterol regulation in the brain.

References

[1] (1) Cardenia V, et al. (2017), J. Steroid Biochem. Mol. Biol. 169, 137-144; Malaguti M, et al. (2019), J. Steroid Biochem. Mol. Biol. 193, 105430.

The impact on human plasma lipidome of patients with colon cancer is stage- and sex-dependent: specific decrease on linoleic acid-containing species

Serena Sadoun (1), Maria Barceló-Nicolau (2), Alice Chaplin (3), Ramón Rodríguez (1), Albert Maimó-Barceló (1), Pedro Santiago (4), Helena Beatriz Ferreira (4), Tânia Melo (4), Rosário Domingues (4), Dora Romaguera (3), Gwendolyn Barceló Coblijn (1)

(1) Health Research Institute of the Balearic Islands (IdISBa) and Research Unit, University Hospital Son Espases, (2) Health Research Institute of the Balearic Islands (IdISBa), (3) Health Research Institute of the Balearic Islands (IdISBa) and Research Unit, University Hospital Son Espases and Consorcio CIBER, M.P. Fisiopatología de la Obesidad y Nutrición (CIBEROBN), Instituto de Salud Carlos III (ISCIII), (4) Mass Spectrometry Center, LAQV-REQUIMTE, Department of Chemistry, University of Aveiro and CESAM - Centre for Environmental and Marine Studies, Department of Chemistry, University of Aveiro

Type of abstract: POSTER

Introduction and Aims:

Colon cancer (CC) is one of the most preventable yet common and lethal cancers. Evidence shows that lifestyle, body composition, and metabolic dysfunction play roles in its aetiology and progression. Research has largely examined tumour biology in isolation from systemic metabolic dynamics, overlooking their potential interrelation. An interesting, yet less studied, phenomenon is that cancer patients often present with dysregulated lipid metabolism and altered plasma lipid profiles. Previously, we demonstrated that patients with CC show decreased content of plasma esterified fatty acids by approximately 60%. To further explore this observation, we analysed plasma at the lipid species level to investigate whether stage-specific alterations could be identified.

Material and Methods:

Patients were recruited at the University Hospital Son Espases (Palma, Spain). The cohort consisted of healthy controls (n=20, 8F/12M) and patients with CC (n=137), classified according to the TNM Staging System: pT1 [n=16, 5F/11M], pT2 [n=17, 7F/10M], pT3 [n=69, 33F/36M], and pT4 [n=35, 15F/20M]). Lipid profile was established using reverse-phase LC-MS. FABP4, insulin, leptin, and resistin plasma concentrations were measured using a multiplex human cytokine magnetic bead panel (Luminex®, R&D Systems). Relevant clinical data on inflammatory biomarkers (fibrinogen and C-reactive protein) were also collected.

Results:

The results showed a profound and consistent decrease in plasma linoleic acid (LA)-containing species, particularly affecting cholesteryl esters and phospholipids at absolute and relative levels. Remarkably, the decrease was TNM-stage and sex-dependent, being more pronounced in men than in women, which could partly account for the well-established higher incidence of CC in men. Unexpectedly, the triacylglycerol class was barely affected. These observations concur with stage-dependent changes in FABP4, insulin, leptin, and systemic inflammatory markers. A subset of patients with CC were sorted using the Consensus Molecular Subtype (CMS) classification, solely based on tumour differential transcriptomic profile: CMS1 (MSI immune, n=8 [6F/2M]), CMS2 (canonical, n=17 [9F/8M]), CMS3 (metabolic, n=5 [3F/2M]), and CMS4 (mesenchymal, n=18 [7F/11M]). CMS4, characterized by strong stromal infiltration and a poor prognosis, showed the most altered content in LA-species.

Conclusions:

Altogether, plasma lipidome proved to be highly sensitive to the presence and progression of CC, while providing unique information regarding the potential underlying mechanisms of the disease.

Development of an Automated Lipidomics System for Reliable Data Accumulation Toward Molecular Precision Medicine

Kazutaka Ikeda (1), Hirofumi Fujimoto (2), Kenta Matsumoto (2), Kohta Nakatani (3), Masaki Yamada (2), Yoshihiro Hayakawa (2), Takeshi Bamba (4)

(1) Kazusa DNA Research Institute, (2) Shimadzu Corporation, (3) Center for Research Promotion, School of Medicine, Niigata University, (4) Medical Institute of Bioregulation, Kyushu University

Type of abstract: POSTER

Small molecule omics analysis using the ZenoTOF 8600 system

Paul Baker (1), Robert Proos (1), Rebekah Sayers (1)

(1) SCIEX

Type of abstract: POSTER

Impact of Dietary Supplementation with Docosahexaenoic Acid in Regio- and Enantiopure Triacylglycerols on Metabolomic Profiles of Liver and Brain in Rats

Baoru Yang (1), Pariyani Raghunath (2), Yumei Zhang (3), Guðmundur Haraldsson (4)

(1) University of Turku, (2) University of Turku, Department of Life Technologies, (3) School of Public Health, Peking University, (4) Science Institute, University of Iceland

Type of abstract: POSTER

N-3 polyunsaturated fatty acids (n-3 PUFAs) play important roles in cognitive functions. In natural sources of N-3 PUFAs are mainly present in the form of glycerolipids, with triacylglycerols (TGs) being the dominating lipid class in marine species. Previous research has shown that differ marine resources may differ in the positional distribution of n-3 PUFAs in TG molecules. However, there is a lack of knowledge on how such difference in positional distribution of n-3 PUFAs may lead to potentially different the metabolic impact. Therefore, the aim of this research was to study the impact of regio- and stereo-specific positioning of n-3 PUFAs in dietary TGs on the metabolomics profile in liver and brain in rats.

Rats in a state of mild n-3 PUFA deficiency were fed daily with 360 mg structured TGs containing DHA (docosahexaenoic acid) at sn (stereospecific numbering)-1, 2, or 3 positions and stearic (18:0) at remaining positions, or an equal amount of tristearin for 5 days. Groups fed with n-3 deficient diet and normal n-3 adequate diet were included as controls. The metabolic profiles of the brain and liver were studied using nuclear magnetic resonance (NMR)-based metabolomics. Several metabolites of significance in membrane integrity and neurotransmission, and glutamate, in particular, are significantly lower in the brain of the groups fed with sn-1 and sn-3 DHA compared to the sn-2 DHA group. Further, the tristearin and DHA groups show a lower lactate level compared to the groups fed on normal or n-3 deficient diet, suggesting a prominent role of stearic acid in regulating energy metabolism. This study sheds light on the impact of stereospecific positioning of DHA in triacylglycerols and indicated a potential role of dietary stearic acid on metabolism in the brain and liver.

Acknowledgement

The authors gratefully acknowledge the funding provided by the Research Council of Finland (Decision No.356891), the Business Finland (Decision No. 1675/31/2023), the Finland-China Food and Health Network, and the China Scholarship Council.

References

[1] Pariyani R, Zhang Y, Haraldsson G, Chen K, Linderborg K, Yang B. Metabolomic Investigation of Brain and Liver in Rats Fed Docosahexaenoic Acid in Regio- and Enantiopure Triacylglycerols. Mol. Nutr. Food Res. 2024, 68, 2300341 https://doi.org/10.1002/mnfr.202300341

Malassezia-mediated Microbe/Host Interaction: Clinical Insights via Oxylipin Lipidomics

Luca Antonio Plado (1), Bala Davient (2), Stelle Tan (3), Nathania Chan (2), Cheryl Leong (2), Shi Mun Lee (2), Peter Imre Benke (4), Federico Torta (5), Alexandra Kendall (1), Anna Nicolaou (1), Thomas L Dawson Jr (6)

*(1) The University of Manchester, (2) Institute of Singapore (SRIS), (3) Institute of Singapore (SRIS),, (4) National University of Singapore, (5) National University of Singapore and Duke-NUS Medical School, (6) A*STAR Skin Research Labs (A*SRL) and Medical University of South Carolina*

Type of abstract: POSTER

Oxylipins, oxygenated derivatives of polyunsaturated fatty acids (PUFA), are common fungal-derived intermediates and play important roles in skin immunology, keratinocyte biology, and inter-kingdom signalling. While octadecanoids and eicosanoids are consistently detected on superficial skin, their biological role and origin remain unclear (1). Malassezia are lipid-dependent yeasts that dominate the human skin mycobiome and are implicated in inflammatory skin conditions, including dandruff and seborrheic dermatitis. Beyond their pathogenic associations, Malassezia are increasingly recognised as active metabolic contributors to skin homeostasis.

Our in vitro studies have shown that Malassezia can secrete octadecanoids, including the dihydroxy fatty acids 9,10-dihydroxyoctadecenoic acid (9,10-DiHOME) and 9,10-dihydroxyoctadecanoic acid (9,10-DiHODA) (approximately 7 and 17 ng per 1x105 colony-forming units, respectively). Other octadecanoids, such as trihydroxyoctadecenoic acids (TriHOME), are also secreted at lower concentrations. Using stable isotope tracing, we confirmed that oleic and linoleic acid are metabolised by Malassezia, forming 9,10-DiHODA and 9,10-DiHOME, respectively. Furthermore, we have shown that secretion of oxylipins by M. restricta and M. globosa, two Malassezia species commonly detected on healthy skin, is significantly higher than that of species not commonly detected, such as M. furfur. These findings suggest Malassezia may contribute to human skin-surface oxylipins. Indeed, our previous work comparing the skin of pre-pubescent children and adults, including pre- and post-menopausal females, revealed that M. restricta abundance positively correlates with skin 9,10-DiHOME, suggesting a potential age- and microbe-dependent contribution to skin oxylipins (2).

To assess the microbial origin of scalp skin surface oxylipins through targeted Malassezia suppression, we performed a clinical intervention study and quantified both oxylipins and microbes, before and after an antifungal treatment. We recruited 67 healthy adults (34 males, 33 females; aged 21-65 years). Microbes were harvested by swabs, and oxylipins were collected by ethanolic scalp washes, before and after treatment with an antifungal shampoo (1% selenium disulfide, an effective inhibitor of Malassezia). Oxylipins (37), including PUFA-derived mono-, di-, and poly-hydroxy, epoxy, and ketone oxygenated derivatives, were quantified using targeted lipidomics by UPLC/ESI-MS/MS. The abundance of key scalp commensals (M. restricta, M. globosa, Cutibacterium acnes, and Staphylococcus epidermidis) was measured by quantitative PCR.

This work will clarify the contribution of Malassezia to oxylipin production on human scalp and their role in skin health relevant host-microbe signalling. As fungal oxylipins could mediate inflammatory and barrier-related processes leading to the development of itch, flaking, and irritation, this study may highlight pathways underpinning the interaction between skin and microbiota in common skin disorders.

References:

(1) Nicolaou A, Kendall AC. (2024) Pharmacol Ther, 260:108681. PMID: 38897295

(2) Pagac M.P, Davient B, Plado, L.A, et al. (2025) npj Biofilms Microbiomes, 11,13. PMID: 39800795

Untargeted lipidomic analysis of liver samples: changes in a mouse model of anaphylaxis

Natalia Rzetecka (1), René Nauhaus (2), Sergio Fernandez-Bravo (3), Coral Barbas (2), Vanesa Esteban (4), Irene de María-Camacho (3), Alma Villaseñor (2)

(1) Department of Inorganic & Analytical Chemistry, Poznan University of Medical Sciences; Centro de Metabolómica y Bioanálisis (CEMBIO), Faculty of Pharmacy, Universidad San Pablo-CEU, CEU Universities, (2) Centro de Metabolómica y Bioanálisis (CEMBIO), Faculty of Pharmacy, Universidad San Pablo-CEU, CEU Universities, (3) Department of Allergy and Immunology, IIS-Fundación Jiménez Díaz, UAM, (4) Department of Allergy and Immunology, IIS-Fundación Jiménez Díaz, UAM; Faculty of Medicine and Biomedicine, Alfonso X El Sabio University

Type of abstract: POSTER

Introduction and Aims:

Anaphylaxis is the most severe type of allergic reaction, characterised by a rapid onset and potentially fatal consequences such as hypotension, vasodilation, bronchospasm, erythema, arrhythmia, or death. Reactions to factors such as insect stings, drugs and foods remain a serious problem and are becoming more frequent. Various biological organs such as sking, lung or brain can be affected. However, how the liver can be affected in anaphylaxis is still not known. The liver plays a pivotal role in lipid metabolism, for this reason, it is crucial to examine the changes in the liver's lipidome that occur during an anaphylactic reaction (1). The aim of this study was to detect significant lipidomic differences in the liver between three groups of mouse models: control, sensitized, and anaphylaxis.

Material and Methods:

In our study, mice were sensitized epicutaneously during 6 weeks with the major peach allergen in the Mediterranean area, Pru p 3, and animals were challenged one week after. Anaphylaxis was assessed by measuring the drop of temperature after challenge. Liver samples were taken and studied within groups: sensitized (n=6), with anaphylaxis (n=6), and control (n=6). Lipid extraction was performed using methanol containing internal standards and MTBE and samples were analyzed by untargeted lipidomics in a UHPLC system coupled to a quadrupole time-of-flight (QTOF) mass spectrometer. Data were acquired in both ionization modes in separate runs: positive ionization (ESI+) and negative ionization (ESI-). A highly reliable in-house lipid database (2) was used to extract lipids from the acquisition data files using Profinder. After quality assurance, the data were normalized by the total useful signal (TUS). ANOVA or Kruskal-Wallis tests were performed based on the distribution of each metabolite for the univariate statistical analysis.

Results:

The targeted profinder analysis yielded a total of 265 lipids in positive and 75 lipids in negative ionisation modes. The following lipid categories were found: fatty acyls, glycerolipids, glycerophospholipids, sphingolipids, and sterol lipids. The PCA model of these data shows clustering of QC samples, indicating good quality of the data. We identified significant differences between groups: control-sensitized (32 lipids), control-anaphylaxis (30 lipids), and sensitized-anaphylaxis (13 lipids). Most of the significant lipids had higher levels in the sensitized and anaphylaxis groups compared to the control. Especially triacylglycerols, glycerophosphocholines, sphingolipids and fatty acids seem to play an important role.

Conclusions:

The results indicate major differences between the sensitised and anaphylaxis groups compared to the control group. These findings provide key insights into the lipid metabolism in the liver of sensitized and food anaphylaxis mice, which may help to understand the underlying mechanisms.

References

[1] Fernandez-Bravo S, et al. (2024) J Allergy Clin Immunol. 154(3), 827-832. PMID: 38718948

[2] Martínez S, et al. (2024) J Lipid Res., 65(11):100671. PMID:393957